A COMPARISON OF IGA ANTIBODY LEVELS IN CARIES-RESISTANT AND CARIES-SUSCEPTIBLE CHILDREN

by

Paul Todd Rose

Submitted to the Graduate Faculty of the School of Dentistry in partial fulfillment of the requirements for the degree of Master of Science in Dentistry, Indiana University School of Dentistry, 1993.

Thesis accepted by the faculty of the Department of Pediatric Dentistry, Indiana University School of Dentistry, in partial fulfillment of the requirements for the degree of Master of Science in Dentistry.

_____

Richard L. Gregory

_____

David R. Avery

_____

Brian J. Sanders

_____

Ronald A. Branca

_____

Christopher V. Hughes

Chairman of the Committee

Date_____

ACKNOWLEDGMENTS

Our Lord has blessed me. In His undoubting wisdom He has watched over me and filled by life with many special people. People who have guided and helped me to reach my goals. People who have supported me so that I could accomplish whatever I desired.

This research effort is dedicated to Rachel Andrada without whose love, understanding, and patience this thesis could not have been completed. Rachel has eased my fears, comforted my disappointments, and inspired me to always give my best effort. Rachel is my best friend, my partner, my love, and my wife.

I would like to thank my parents for their support throughout my life. They have always provided love, support and financial backing for all my endeavors and endowed me with an understanding of what is truly important in life.

Special thanks to Dr. Christopher Hughes, my mentor, for his many words of wisdom and guidance. Dr. Hughes kept me on the straight and narrow and never lost sight of the true goal. Dr. Hughes was not only responsible for introducing me to all the right people

but also for providing me with the motivation to see my thesis to the end.

Also, special thanks to Dr. Richard Gregory for providing laboratory space and supplies, financial backing, toys for the children, editorial and moral support. It has been my privilege to work with Dr. Gregory, and I have grown to respect his expertise.

Thank you to my Research Committee members: Dr. Christopher Hughes, Dr. Richard Gregory, Dr. Brian Sanders, Dr. David Avery, and Dr. Ronald Branca.

Thank you to Linda Gfell for all the help in the lab and for proving that even a dental resident could be trained to perform the ELISA technique.

Thanks to all the faculty in the Department of Pediatric Dentistry at the Indiana University School of Dentistry for providing the patient population and helping in the subject selection process.

Thanks to Alane and Janet at Riley Children's Hospital Dental Clinic for their extra help in patient selection.

Thanks for the support provided by USPHS grant DE 07318.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

INTRODUCTION

Secretory immunity is believed to play a role in natural resistance to dental caries. Although dental caries has dramatically decreased in children in the United States, there remains a population of caries-susceptible children even in fluoridated communities. Previous studies have shown a positive correlation between salivary immunoglobulin A (sIgA) antibody levels to *Streptococcus mutans* and caries resistance in adults. The goal of this research study was to investigate the role of the immune response in natural protection against caries in children by examining and comparing the amounts of IgA antibodies to *S. mutans* among caries-resistant and caries-susceptible children. This was performed by collection of whole and parotid saliva, analysis of antibody activity by enzyme-linked immunosorbent assay and bacterial numbers by spiral plated cultures, and by comparison between the caries-resistant and caries-susceptible subjects.

REVIEW OF LITERATURE

Despite significant advances in dental caries prevention, dental decay continues to be a problem throughout much of the world. The preventive measures against dental caries in developed countries have proven to be quite effective, reflected in a continued decrease in caries prevalence over the past 20 years. The National Institutes of Health reported as recently as 1980 that the mean number of decayed, missing, and filled permanent tooth surfaces in United States adolescent population has increased from 5.41 for 13-year-olds to 11.04 for 17-year-olds. However, it is evident that preventive pit and fissure sealants and the use of fluorides continue to be relied upon for caries control. According to Nowak and Crall[1] other factors such as dietary habits and motivation continue to play major roles in caries rate. Smith[2] and Diesendorf[3] report that in most undeveloped countries dental decay remains the most common form of human disease. The main cause for continued dental disease is simply the economic barriers that prevent the provision of dental health care and education to the population of the these undeveloped countries. These economic barriers include the finances to start the

preventive care programs such as water fluoridation, preventive dentistry, and nutritional education. Another report by Smith[4] indicates that these economic barriers coupled with increased sugar intake especially in the younger population have actually led to increasing caries rates in developing countries. Data from the World Health Organization estimate that the average 14-year-old in developing countries has four decayed teeth.[5] Therefore, it is imperative that more cost-effective means of preventing dental caries must be developed. This can be accomplished by continuing research in caries prevention in the area of caries immunization.

The role of *Streptococcus mutans* in dental caries

It has long been known that microorganisms were involved in disease, but it was not until the 1880s that Miller's[6] experiments placed dental caries into the category of a bacterial disease. Miller demonstrated that, in the presence of carbohydrate, salivary bacteria could demineralize tooth enamel. He, therefore, postulated that the etiologic agents of dental caries were bacteria and that their acidic and proteolytic products caused destruction of the

mineral and organic components of the teeth. Miller isolated several types of bacteria that were capable of producing large amounts of acid and concluded that no single species was responsible for dental caries and that any bacteria capable of producing acid could cause decay.

The involvement of *Streptococcus mutans* in the dental caries process was first proposed almost 70 years ago. In 1924 Clarke[7] isolated *S. mutans* from human carious lesions and recognized its potential role in the etiology of dental caries. Clarke named the microorganism *S. mutans* because the isolated organisms had a tendency to be somewhat pleomorphic, that is, possessing either cocci chains or short rods. He proposed that the acidogenic and aciduric properties of *S. mutans* may be responsible for dental decay.

The early report by Clarke was mostly ignored by the scientific community for 30 years. Later, with the development of gnotobiotic animal technology, experiments confirming the bacterial etiology of dental caries were conducted. In 1955 Orland and associates[8] demonstrated that germ-free rats fed high sucrose diets were caries-free. When subsequently monoinfected with

streptococcal species, these animals developed dental caries. Orland's experiments proved the involvement of bacteria by demonstrating that in the absence of bacteria, dental caries did not develop. In 1960 Keyes[9,10] performed experiments that established that dental caries was an infectious disease. He treated hamster dams with antibiotics during pregnancy and lactation and produced caries-inactive offspring. These caries- inactive pups failed to develop extensive lesions unless they were caged with animals exhibiting rampant decay or were supplied with fecal material from carious animals and consequently became orally infected due to coprophagous habits. Later, Fitzgerald and Keyes[11] were able to isolate certain streptococci from carious lesions in hamsters. They innoculated the mouths of the caries-free hamsters with these bacteria and the hamsters exhibited rampant decay. Their work further suggested that certain strains of oral streptococci may be responsible for dental decay, the principal being *S. mutans.*

Although these extensive studies suggested that *S. mutans* had an etiologic role in dental caries, it was not until the 1970s that Loesche and others provided definitive evidence that demonstrated that *S.mutans* was the etologic agent of human dental caries.

Loesche and associates[12-14] used associative, longitudinal, virulence, and response-to-treatment studies to provide this evidence, and the role of *S. mutans* in human dental decay has since stimulated an enormous research effort.

Studies by Bratthall[15] and by Perch et al.[16] have divided the *S. mutans* isolates into eight serotypes (*a-h*) based on differences in the cell wall carbohydrates. These serotypes exhibit cross-reactivity, especially between serotypes *a*, *d*, *g*, and *h* and serotypes *c*, *e*, and *f*. DNA hybridization studies by Coykendall[17] divide these isolates into four genetic groups based on the percentage of guanine and cytosine (G + C in the DNA). Mutans streptococci currently have the following species designations: human isolates that resemble Clarke's originally identified strain are classified as *S. mutans* and represent serotypes *c*, *e*, and *f* (36 to 38 percent G + C), with *S. mutans* serotype c being the most prevalent mutans streptococci isolated from human dental plaque. Loesche[11] reports that *Streptococcus sobrinus* consists of serotypes *d*, *g*, and *h* human isolates (44 to 46 percent G + C). *Streptococcus cricetus* are the

serotype *a* isolates (42 to 44 percent G + C) and *Streptococcus rattus* are serotype *b* isolates (41 to 43 percent G + C).

The probable role of *S. mutans* in dental decay prompted investigators to conduct further examinations of immunity to *S. mutans* in caries susceptible and caries resistant individuals to determine the extent to which an immune response to this organism plays a role in natural resistance to caries.

Immune response studies

Michalek and Childers[18] reviewed that secretory Immunoglobulin A (sIgA) has been identified as the principal immunoglobulin isotype present in external secretions and as the main humoral element of the secretory immune system. SIgA is known to neutralize viruses, bacterial exotoxins, and enzymes that contribute to disease processes and to inhibit the attachment and adherence of oral bacteria to epithelial and tooth surfaces. Thus, the induction of a sIgA antibody response to *S. mutans* antigens it would seem helpful in the development of a caries immunization.

Different routes of immunization are believed to exist from which an antibody response could be induced by *S. mutans* antigens.

One route postulates that the humoral immune response of serum IgG to *S. mutans* antigen may play an important role in natural immunity to dental caries. The basis of this postulation is that there is an exposure of blood to the oral cavity at the time of tooth eruption and then following eruption via crevicular fluid. In 1970 Attstron and Egelberg[19] suggested that crevicular fluid may be the vehicle of IgG antibody-mediated reductions in experimental dental caries in animals immunized with *S. mutans* antigens, and then in 1986 Krasse et al.[20] suggested that immune components from serum can enter the oral cavity during the eruption process. In order to provide mechanisms for this, Riott and Lehner[21] theorized that antigens on the surface of microorganisms are phagocytized by macrophages in sites other than the oral cavity. This allows antigenic materials to be processed and become associated with the surface of the macrophages and then to be presented to B and T lymphocytes. The B-lymphocytes are activated and, with assistance from helper T-lymphocytes, remain in lymphoid tissue in the oral cavity and differentiate into plasma cells which in turn produce antibodies. This results in the release of specific IgG antibodies in

the oral cavity that are capable of binding to antigen. The plasma cells only survive two to three days but memory B-lymphocytes, which are derived from the B-lymphocyte population, may be present for several years.

However, one report by Bienenstock et al.[22] and another by Mestecky et al.[23] have provided considerable evidence that indicates that exposure of lymphocytes to the oral cavity is not the major pathway leading to a secretory immune response. Secretions of glands such as mammary, salivary, and lacrimal contain sIgA antibodies to antigens that have never been present in the gland. Since sIgA in secretions is locally produced by plasma cells in the gland, it follows that precursors of IgA B cells home to these glands after prior stimulation in IgA inductive sites. This supports the postulation that a common mucosal immune system exists. According to the postulation antigens are swallowed or inhaled, in this case the *S. mutans* bacteria, then taken up by specialized phagocytic antigen-presenting epithelial cells (microfolding or M cells) which in turn stimulate IgA precursor B and regulatory T cell activity in gut-associated lymphoreticular tissue (GALT) (i.e. Peyer's Patches), and bronchus-associated lymphoreticular tissue

(BALT), respectively. These cells then migrate through the circulation and lymphatics to distant mucosal sites including the lamina propriae of the gastrointestinal, respiratory, and genitourinary tracts and the salivary, lacrimal and mammary glands and differentiate into plasma cells. The plasma cells are found in subepithelial areas where they produce and actively secrete antibodies. Secretory component (SC), which serves as the IgA receptor on secretory epithelial cells, selectively binds to the J-chain containing IgA. SC is produced by the secretory epithelial cells that line the intestinal tract and acinar and ductal cells of the secretory glands. The SC then acts as a transporting protein to translocate the IgA:J chain:SC complex from the distal surface of the epithelial cell into an external secretion (Figure 1). SC also makes sIgA more resistant to the nonspecific proteolytic enzymes of the GI tract.

External secretions containing sIgA can neutralize enzymes, toxins and viruses as well as prevent the absorption of antigens from the GI tract. SIgA also has been found to affect bacterial adherence to the epithelial lining of the mucosal surfaces of the gastrointestinal, respiratory, and genitourinary tracts.

The initial evidence for the existence of a common mucosal immune system came from studies in animals. Rudzik[24] showed that lymphoid cells from Peyer's patches of rabbits, when injected into irradiated allogeneic recipients, repopulate the lamina propria of the intestine with IgA-producing plasma cells of the donor type. It was also shown that BALT lymphocytes could do the same. Michalek et al.[18] recently demonstrated B cells in the peripheral blood circulation with the potential to produce IgA antibodies to oral vaccine antigens, which has provided additional evidence for the existence of a common mucosal immune system in humans. Much remains to be done to determine the mechanisms involved in the induction and regulation of mucosal responses and to establish the optimal dose and form of oral antigens to be used in humans for induction of protective sIgA repsonses to microbial pathogens, including oral pathogens.

Immunization studies

With the establishment of a bacterial etiology for decay, Wagner[25] explored the possibility that the immune response plays a

role in natural protection from dental caries. Building upon the work of earlier investigators, Wagner reported in 1967 that gnotobiotic rats monoinfected with a strain of *Streptococcus faecalis*, either by oral inoculation or parental injection of the homologous bacterium in adjuvant, were immunized against dental caries. This immunization resulted in the virtual elimination of cariogenic activity seen in the non-immunized gnotobiotic control rats. The immunized and protected rats had higher anti-*S. faecalis* serum and higher salivary agglutinin titers and fewer *S. faecalis* in their saliva than did their non-immunized counterparts. His findings suggested that antibody is produced against the cariogenic bacterium and is secreted into the serum and the saliva. In the immunized gnotobiotic rat challenged with *S. faecalis*, the incidence of caries was reduced. This investigation was conceived as a first step to test the possible protection conferred by immunization and was designed to examine the possibility that there were differences in serum antibody levels against known cariogenic streptococci in a caries-free and a caries-rampant human population.

In the mid 1970s McGhee and associates[26] and Taubman and Smith[27] provided further evidence that immunization procedures in

rats were possible. *S. sobrinus* whole cells were injected into the salivary glands of rats resulting in salivary antibodies that correlated with protection against dental caries. These studies provided evidence that site-restricted (salivary) IgA responses can be induced by local immunization and were the first to show a direct correlation between the appearance of salivary IgA anti-*S. sobrinus* antibodies and protection against *S. sobrinus*-induced caries formation. Taubman and Smith's[28-32] work further suggested that *S. sobrinus* glucosyltransferase (GTF) was an effective antigen in rodents for inducing caries immunity. The salivary IgA anti-*S. sobrinus* GTF antibodies correlated with protection against carious lesions from *S. sobrinus* as well as *S. mutans*. Taubman and Smith[33] also showed that the induced salivary IgA anti-GTF antibodies were shown to block *S. sobrinus* plaque formation *in vitro*. Van deRijn[34] expressed a concern that a potential existed for cross-reactivity of *S. mutans* antibodies to human muscle, kidney and heart tissues. However, building on the suggestions from Taubman and Smith's studies, Michalek et al.[35-37] demonstrated that the oral administration of *S. sobrinus* whole cell antigen to gnotobiotic rats

induced a secretory IgA immune response and that the appearance of specific salivary IgA antibodies correlated with caries immunity without a serum response which would minimize the potential pathology of human cross-reactive antibodies.

Further studies with rats performed by Michalek et al.[37] examined different types of immunogens from *S. mutans.* It was found that particulate antigens were more effective oral immunogens in inducing salivary immune responses than soluble forms. Several other studies examined the use of particulate antigens that were placed directly into the stomachs of rats. These studies, performed by Cox,[38] Kiyono,[39] and Ebersole,[40] agreed that when particulate antigens were placed directly into the rats' stomachs, there was a higher secretory immune response as compared to when soluble forms were given by the oral route due to the more efficient uptake and processing of particulate antigens by GALT.

Michalek et al.[18] next showed that the use of liposomes to present *S. mutans* antigens to GALT induced higher levels of response in rats than just the antigens alone. Morisaki et al.[41] then

investigated the possibility of using soluble adjuvants such as muramyl dipeptide and peptidoglycan to enhance secretory immune responses when given orally with streptococcal antigens, and they found an enhanced salivary IgA immune response in rats. Taubman et al.[32] also showed that intragastric immunization with soluble adjuvants resulted in an enhanced response. These and other experiments provided evidence that soluble adjuvants are effective orally and that they augment IgA immune responses to *S. mutans* antigens.

Caries risk

Loesche[42] reported that the presence of *S. mutans* in a mouth is not a useful diagnostic criterion because almost all individuals harbor *S. mutans* on their teeth and in their saliva. It is the level of colonization of the teeth that determines the risk of decay. He suggested that the most appropriate way to determine numbers of *S. mutans* was by quantitative culturing of plaque from indicator teeth, such as occlusal fissures of first molars, or by culturing the saliva. He reported that since saliva is easier to handle and with the availability of a selective medium for *S. mutans* information on the

salivary levels of *S. mutans* can be routinely obtained by any hospital microbiology laboratory. Bratthall[43] has also reported the use of a semiquantitative method within the dental office using tongue blades and selective media to identify high-caries risk groups.

Klock and Krasse[44] reported that children who have salivary *S. mutans* numbers above 1.0E+06 cells/ml should be considered as being a high-caries risk. Loesche[6] expanded on this information by adding that single occlusal fissures can be colonized from the saliva by as few as 3.0E+03 *S. mutans*/ml of saliva, whereas about 4.5E+04 *S. mutans*/ml are needed to reliably colonize a smooth surface. This would suggest that if the laboratory report for *S. mutans* was in the vicinity of 1.0E+03 ml to 1.0E+04/ml, the patient would not be at high risk for caries, but if the value were over 1.0E+06/ml, he would be at high risk. Loesche concluded that laboratory cultures should be able to recognize those patients at high risk who need concurrent anti-*S. mutans* therapy in conjunction with their restorative treatment.

Loesche[12] reported in another study that it is important to determine the caries-active versus the caries-inactive and caries-free patients. He defines caries active as at least one decayed tooth surface (D>1), caries inactive as no decayed tooth surfaces (D=0), and caries free as no decayed, filled or missing (because of decay) tooth surfaces (D=0). He felt that patients should be divided by the magnitude of their current or past caries experience. For instance, when a DMFS score of five was used as the cut off point between low or high initial caries scores, a negative correlation between lactobacillus numbers and initiation of decay was suggested in the low-caries active subjects, whereas a highly positive correlation between *S. mutans* numbers and initiation was found in the high caries-active children.

Natural immunity

The major emphasis of most caries immunization investigations involves the attempt to enhance the natural immunity to human dental caries. Since the cause of dental caries is a result of the chronic and cyclic process of demineralization, it is reasonable that if antibodies can block attachment or interfere with

bacterial cell function then their enhancement may help prevent dental caries. IgA constitutes the majority of immunoglobulins present in the oral cavity and in the entire body and consists of two subclasses, IgA1 and IgA2. Delacroix et al.[45] identified that IgA1 makes up approximately 60 percent and IgA2 makes up approximately 40 percent of the total IgA. Smith and Taubman[46] have reported that adult parotid saliva contains 30 to 160 μg/ml of IgA and is almost entirely secretory IgA. IgM and IgG are also present but in significantly lower concentrations. Adult whole saliva is usually in the same proportions as infant whole saliva except that minor salivary glands contribute more IgM and IgG in infants. It must also be noted that Smith, King, and Taubman[47] observed that essentially all IgA in whole saliva of predentate infants is of secretory origin, that adult salivary IgM/IgA proportions are achieved early in life, but that, at least in some infants, the salivary IgA subclass levels are still changing during this time.

However, it is well known that salivary IgA is not present at birth but develops during the first months of life to become the

dominant immunoglobulin. As reviewed by Michalek and Childers,[18] initial protection is believed to be provided in three ways: nonimmune factors such as epithelial barrier and peroxidase enzymes; maternal, placentally transferred IgG antibodies; and IgA antibodies in breast milk. It is believed that the immune response to cariogenic organisms may develop during bacterial colonization and eruption of deciduous teeth in the human infant. Kohler and Bratthall[48] have reported that the oral cavity of the infant becomes rapidly colonized, primarily by organisms derived from the mother. Carlsson[49] and colleagues have followed a group of children from birth to 5 years of age in order to determine the progression of colonization of oral microorganisms. First to appear in the oral cavity soon after birth is *Streptococcus salivarius*. *Streptococcus mitis* dominates by 1 to 5 months of age followed by *Streptococcus sanguis* when the first teeth erupt at about 6 months of age. Mutans streptococci are usually not found in appreciable levels until the second or third years of life. This colonization progression provides guidelines by which the selection of streptococcal antigens to use in

probing secretory immune responses in the saliva of the infant can be made.

Camling and Kohler,[50] as well as others, have reported that between the age of 1 and 5 years, salivary IgA antibody has been detected to several mutans streptococcal species, including *S. mutans*. Smith[51] reports in a longitudinal study that the IgA antibody level to *S. mutans* continues to increase from 5 months to 3 years of age and Gahnberg[52] confirms similar findings in the age group from 2 to 48 months of age.

The immune response of infants is important in determining the pattern of colonization of microorganisms in the mouth. Ivanyi and Lehner[53] investigated the comparative maternal-fetal immune responses to *S. mutans*. Their investigations revealed maternal IgG but not IgM isotype of antibodies to *S. mutans* in neonatal blood and that sensitization of neonatal lymphocytes to *S. mutans* occur *in utero*. As mentioned earlier, it is believed that systemic humoral and cellular immune components enter the mouth predominantly through the gingival crevice (Attstrom and Egelberg[19]). This led researchers to believe that there may be a critical timing between

the eruption of deciduous teeth and the physiological clearance of maternal antibodies about 5 months after birth, as well as the duration of sensitization of lymphocytes, which may influence the colonization of plaque bacteria.

Some researchers have even suggested that an effective way to prevent dental caries may be to delay the initial colonization of *S. mutans* in the human mouth. Camling, Gahnberg and Krasse[54] hypothesized that several factors could delay colonization such as exposure to low numbers of *S. mutans*, a low sucrose diet, or salivary factors such as secretory IgA. Camling and Kohler[50] further suggested that IgA antibody activity to *S. mutans* specific serotype *c* antigen and *S. mutans* serotype *c* whole cells was significantly higher in children with more than two decayed or filled surfaces as compared to children who were caries free.

Aaltonen and associates[55] presented data suggesting that the amount of anti-*S. mutans* antibodies occurring in maternal serum and saliva is influenced by the maternal antigenic load. They found that if mothers had dental treatment during pregnancy their *S. mutans* numbers decreased and that there was a correlation with

their decreased serum levels of antibodies to *S. mutans*, which resulted in increased caries in their breast-fed infants. They proposed two explanations for these findings: differences in transplacental passage of IgG antibodies and/or differences in postpartum transfer of *S. mutans* to the infant.

Breast milk plays an important role in the passive transfer of antibodies to the newborn. Mellander, Carlsson and Hanson[56] reported little sIgA is found in the infants saliva in the first few months after birth. They found that the main source of IgA for the baby comes from the mother's breast milk and it is not until the age of about 12 months that adult levels of sIgA are reached. It has been estimated by Brandtzaeg[57] that as much as 1 g/day of immunoglobulin is received by infants from breast milk. It is believed that breast milk IgA antibodies interfere with mucosal binding of microorganisms and prevent exposure to food proteins. Tank and Storvick[58] found that breast-fed infants were less susceptible to caries; however, it must be noted that there is a conflicting report by Camling et al.[59] as to how important a role the

protection provided by breast milk antibodies plays in caries prevention.

Recent human immunization studies

Several human studies have been attempted including the Hughes et al.[60] report of the use of a purified surface protein of *S. mutans* to stimulate a systemic immune response to *S. mutans* antigen, which indicated no increase in the level of cross-reactive antibodies to human tissues. Then, the first protection from *S. mutans* colonization by oral immunization in humans was provided by Gregory and Filler[61] who demonstrated that the ingestion of a vaccine containing killed *S. mutans* induced increased levels of specific sIgA antibodies to *S. mutans* cells and two cell surface proteins, GTF and surface antigen I/II, in tears, saliva, colostrum and milk. These investigations provided additional evidence for the existence of a common mucosal immune system in humans and of memory in this system. However, Gahnberg and Krasse[62] as well as Cole et al.[63] were unable to demonstrate an elevation in salivary IgA antibody levels in humans following oral administration of

mutans streptococcal antigen but noted that there was a decrease in the level of implanted mutans streptococci following immunization with mutans streptococcal antigen. Mestecky et al.[64] have recently conducted experiments in which human volunteers were given *S. mutans* whole cells in enteric-coated capsules by the oral route. Using the enzyme-linked immunospot assay (ELISPOT), it was shown that spontaneous antibody-forming cells were present in the circulation within seven days. Most of the cells produced antibodies of the IgA isotype. Peak levels of sIgA anti-*S. mutans* antibodies in external secretions from these subjects were seen by day 21. No increase in the levels of anti-*S. mutans* antibodies was seen in the sera of these volunteers. This provided additional evidence for the common mucosal immune system. More recently, studies by Taubman and Smith[65] provided further evidence for the induction of a salivary IgA immune response in humans following ingestion of a vaccine containing purified GTF from *S. mutans*. M. Russell, Challacombe, and Lehner[66] and R. Russell[67] reported that the surface of *S. mutans* is composed of many different macromolecules, including GTF, fructosyltransferase, protein surface antigens I/II

and III, proteins A, B, C and P1, SpaA, IF and glucan-binding protein, serotype carbohydrates, lipoteichoic acid, and glucans. After immunizing monkeys with antigens I/II and/or A and C from serotype *c* mutans streptococci, Russell[68] observed a protective effect. The protein antigens B, IF, and PI of mutans streptococci appear to share cross-reactive antigens with heart tissue. Of important note was that GTF did not share any cross-reacting epitopes with human tissue and therefore seems to be a potentially significant antigen. The results of this study and of previous work by this group strongly support the potential use of GTF for use in an oral caries vaccine for humans.

Furthermore, data from Taubman and Smith's studies suggest that salivary IgA may play a very important role in the neutralization of *S. mutans* enzymes. Gregory et al.[69,70] reported that *S. mutans* enzymes most affected by saliva include glucose-phosphotransferase, which transports glucose across the cell membrane. The inhibition of the enzyme would result in decreased growth, acid production, and all other metabolic activites of the cell. Another previously identified enzyme neutralized was GTF, an enzyme that synthesizes water insoluble and soluble glucans. If

these water insoluble glucans are not formed, then *S. mutans* exhibits a decrease in adhesive ability, therefore decreasing colonization on tooth structure.

Curtiss et al.[71] have taken a different approach to developing an oral vaccine by using an avirulent Salmonella mutant as a carrier of cloned genes encoding virulence antigens of mutans streptococci. If avirulent derivatives of Salmonella are endowed with ability to produce *S. mutans* cell-surface proteins, the avirulent Salmonella would home to the GALT cells to elicit production of a secretory immune response against Streptococcal proteins and engender protective immunity against *S. mutans*. Curtiss et al.[71] have shown that peroral immunizations lead to stimulation of sIgA against spaA and GTFA *S. mutans* cell surface proteins and are planning to investigate enhancement of immune responses and the occurence of oral tolerance.

Lehner et al.[72] demonstrated that systemic immunization was possible by using purified sa I/II to prevent the colonization of *S. mutans* in the oral cavity and subsequent dental caries development. Lehner et al.[73] provided evidence that serum IgG antibodies gain

access to the oral cavity by transudation through the gingival crevice to effect protection. Furthermore, studies by Challacombe and Lehner[74] suggested a correlation between *S. mutans* antigen and salivary IgA levels in primates, which were given *S. mutans* antigens in their drinking water. It must be noted that systemic immunization approaches are unlikely to be used in humans until the questions about the role of crevicular fluid in dental caries immunity and the potential for cross-reactivity of *S. mutans* antibody with human heart tissue are answered.

Present study considerations

Gregory et al.[70] has also demonstrated that there were significantly higher levels of IgA2, but not IgA1, salivary antibodies to *S. mutans* whole cells in caries resistant than in caries susceptible adult subjects. However, Smith, King, and Taubman[47] noted that fluctuations in proportions of IgA subclass levels have been observed in various age groups. The subclass levels of sIgA for the 7- to 11-year age group have not been well documented and therefore were one of the main foci of this study.

Also, the need for additional studies in this area has been established due to conflicting experimental reports that do not support the protective role for salivary antibodies to *S. mutans*. Riviere[75] reported in 1987 that there were no significant differences in antibody levels in unstimulated whole saliva and dental plaque for any serotype of *S. mutans* between children of different caries experience. However, the potential cross-reactivity of other bacteria absorbing IgA antibody was not considered when whole saliva was used instead of parotid saliva. Also, the study failed to document the method, time of day and flow rate of unstimulated saliva collection. Other factors that need to be addressed by further study include the levels of IgA1 and IgA2 antibodies in saliva and the quantitation of *S. mutans*.

MATERIALS AND METHODS

Demographics

The study involved a total of 41 subjects who were selected from the patient population of the Department of Pediatric Dentistry, Indiana University School of Dentistry. The following guidelines served as the criteria for patient selection: (1) the patients were all between the ages of 7 and 11 years; (2) the patients had essentially negative past medical histories; (3) the patients were not currently taking any medications; (4) the patients were in the mixed dentition stage and had at least four permanent molars and four permanent incisors; (5) each patient's legal guardian signed a written informed consent following an explanation of the procedure, possible risks and complications; and (6) the patients were grouped as either caries resistant or caries susceptible.

The definitions of caries resistance and caries susceptible were based on statistical data from the National Survey of Dental Caries in US School Children compiled by the National Institute of Dental Research.[76] Caries-resistant individuals were classified as

those individuals who presented with no more than one decayed, missing, or filled tooth surfaces. Caries-susceptible individuals were classified as those subjects who presented five or more unfilled decayed tooth surfaces. The number of five surfaces was derived from the US survey data that combines the mean of permanent tooth surfaces decayed, missing, or filled and the mean of deciduous tooth surfaces decayed, missing, or filled, then calculating +2 standard deviations to the 95 percentile level to arrive at 4.76 decayed, missing, or filled surfaces. Due to the large number of individuals with a relatively low history of caries, the 95 percentile level is not much greater than the average number of decayed, missing or filled surfaces as seen from a clinical standpoint.

Clinical sample collection

One pediatric dental resident (Dr. PT Rose) in the Department of Pediatric Dentistry examined the subjects and collected saliva samples. Each subject was seen prior to noon for collection of unstimulated parotid and whole saliva to standardize collection times. Both parotid and whole saliva were collected so that a

comparison of IgA concentrations and antibody levels could be made. Whole saliva was collected by having the subjects expectorate into a 50 ml tube. Parotid saliva was collected by placing a suction adherent apparatus over the parotid duct opening and allowing the saliva to flow, unstimulated, into a collection portal. The saliva was collected for 20 minutes, then both samples were immediately placed on ice and transported to the laboratory. The parotid sample was placed immediately at -20 degrees Celsius (C) until needed. A portion of whole saliva was used to make dilutions for quantitation of microorganisms, and the remaining sample was frozen immediately at -20 degrees C.

Quantitation of *S. mutans*

S. mutans was detected in whole saliva samples from all subjects by colonial morphology. Briefly, unstimulated saliva samples were diluted (1:10 and 1:100) in sterile saline, vortexed for 30 seconds, and spiral plated in duplicate on Mitis Salivarius agar (Difco Laboratories, Detroit, MI) containing 15 percent sucrose (MSS) and Mitis Salivarius agar (Difco) containing 15 percent sucrose and supplemented with bacitracin (0.2 units/ml) (MSSB) for

enumeration of total oral streptococci and *S. mutans*, respectively, after incubation for three days at 37 degrees C in an atmosphere of 5 percent CO_2 in air.[77] The total oral streptococcal number was determined by counting all colonies on each MSS plate and correcting for dilution and spiral plating for each sample. The total *S. mutans* number was determined by counting all *S. mutans* colonies on each MSSB plate and correcting for dilution and spiral plating for each sample.

Preparation of enzyme-linked immunosorbent assay antigens

All 41 subjects' saliva samples were tested by the following enzyme-linked immunosorbent assay (ELISA) tests simultaneously in order to minimize operator variability. For preparation of *S. mutans* whole cell ELISA antigen, *S. mutans* TH16 (serotype *c*) cells were grown for 18-24 hours at 37 degrees C in 5 percent CO_2 in a dialyzed tryptose broth medium (Difco), harvested by centrifugation (10,000 x *g*; 10 minutes) and washed twice in sterile 0.01 M phosphate buffered saline (pH 7.0; PBS). The washed cells were resuspended in PBS containing 0.5 percent formalin and kept for 48 hours at room temperature to kill the bacteria. The killed cells

were washed twice in PBS, and resuspended to an optical density of 0.500 at 660 nm in 0.1 M sodium bicarbonate/carbonate buffer (pH 9.6) for use in the ELISA.

Determination of immunoglobulin concentrations and antibody levels

An ELISA "sandwich" technique was used to determine the concentrations of whole salivary total IgA, whole salivary total IgA1, whole salivary total IgA2, and parotid salivary total IgA. One hundred μl of unlabeled goat anti-human IgA (Cappel) that had been previously diluted to 1 μg/ml with carbonate buffer containing azide was added to the inner wells of flat-bottomed polystyrene microtiter plates (Flow) and allowed to incubate at 37 degrees C for three hours. The plates were then washed with Tween saline three times and 200 μl of human serum albumin (Sigma) in carbonate buffer diluted to 10 μg/ml was added to each well to block unreacted sites and the plates were incubated at 25 degrees C overnight. The saliva samples were diluted (1:10), vortexed for 30 seconds, and centrifuged for five minutes at 1,200 x *g*. An internal control was used to control interplate variability and consisted of

aliquots of a large pool of unstimulated saliva from one subject not in the study. The plates were washed three times with Tween saline and 100 μl of the diluted saliva samples (1:10) as well as the whole saliva internal control and the Tween saline negative control were added to the wells in triplicate and incubated at 37 degrees C for one hour. Horseradish peroxide-labelled goat antihuman IgA, IgA1, and IgA2 (Cappel) were diluted (1:1000) for use in their respective wells. The plates were washed three times with Tween saline and 100 μl of the anti-human IgA, IgA1, or IgA2 was added to each well and was allowed to incubate for one hour. The wells were washed three times with Tween saline. One hundred μl of the orthophenylene diamine hydrochloride/citrate/H_2O_2 buffer was added to each well and allowed to react at room temperature for 25-30 minutes. The reaction was stopped by adding 100 μl of 2 N H_2SO_4 to all of the wells. The amount of color that developed was measured by using the Titertek Multiskan photometer (Flow) at a wavelength of 490 nm. The mean internal salivary control absorbance for each plate was used to standardize unknown saliva

absorbances between plates in order to control for inter-plate variability due to temperature, pipetting and reagent differences.

Levels of whole salivary IgA antibody, whole salivary IgA1 antibody, whole salivary IgA2 antibody, and parotid salivary IgA antibody levels to *S. mutans* TH16 whole cells were determined by ELISA. One hundred μl of the *S. mutans* whole cell ELISA antigen was added to the inner wells of flat-bottomed polystyrene microtiter plates (EIA, Linbro, Flow Laboratories, McLean, VA). The plates were incubated for three hours at 37 degrees C and then washed three times with 0.9 percent NaCl containing 0.05 percent Tween 20 (Tween-saline) to remove unbound antigen. After the final wash, 10 μg/ml of human serum albumin in carbonate buffer was added to each well (200 μl) to block unreacted sites and the plates were incubated at 25 degrees C overnight. The saliva samples were diluted as discussed earlier. The plates were washed three times with Tween saline and the previously optimized dilutions of human whole saliva (1:10) were added to each well (100 μl) in triplicate. The internal control was used by placing the control saliva (1:10) in triplicate on all plates and a Tween saline negative control was placed in triplicate on all plates. The plates

were incubated at 37 degrees C for one hour. The plates were washed three more times with Tween saline. Horseradish peroxidase-labelled goat anti-human IgA heavy chain-specific reagent (Cappel Scientific Division, Cooper Biomedical, Inc., Malvern, PA) was diluted (1:1000), added to the appropriate wells (100 μl) and incubated at 37 degrees C for one hour. The plates were washed three times with Tween saline and 100 μl of citrate buffer (0.4 mg of orthophenylene diamine hydrochloride [Sigma Chemical, St. Louis, MO]/ml in citrate buffer [pH 5.0] containing 0.025 percent H_2O_2) was added to all the wells and reacted at room temperature for 25-30 minutes. Then, 100 μl of 2 N H_2SO_4 was added to each well to stop the reaction. The amount of color that developed was measured in the microtiter plate by using a Titertek Mulitskan photometer (FLOW) at a wavelength of 490 nm. The mean internal salivary control absorbance for each plate was used as discussed as before.

Statistical Analysis

The mean value was calculated by the summation of three ELISA absorbances for each sample and dividing by three. Any

outlying (< or > 2 standard deviations from mean) readings were excluded from this mean so as to take into consideration any false readings. No more than one outlying reading was discarded for any individual sample that was examined using the ELISA technique. It must be noted that the absorbances for levels of total parotid salivary IgA and total whole salivary IgA were converted to semilog concentration equivalents by using standard purified secretory IgA human colostrum standards. Unless otherwise stated, all variances are reported as standard errors of the mean. The BMDP statistical analysis program (BMDP Statistical Software, Inc., Los Angeles, CA) was used to analyze the values for statistical significance by using Student's t test.

RESULTS

Demographics

There were a total of 41 children in this study. The children ranged in age from 7 years 1 month to 11 years 11 months. The population consisted of 29 caucasian subjects and 12 black subjects. Fifteen subjects were female and 26 subjects were male. *S. mutans* could be isolated from all 41 subjects regardless of caries history. The children were divided into two groups according to their caries history: (1) caries resistant (CR); or (2) caries susceptible (CS). Not all children donated enough saliva for every assay. Demographic breakdowns of the subject population are shown in Table I. The mean age (± one standard deviation) of the CR children was 9.78 ± 1.41 years, and the mean age (± one standard deviation) of the CS children was 9.46 ± 1.39 years. The relatively equal mean ages between the two groups ($p > 0.30$) is illustrated in Figure 2. Age selection for the groups was fairly evenly distributed in one-year increments between the 7- to 11-year range as shown for the CR and the CS subjects in Figure 3.

The CR children had 0.33 ± 0.48 (mean ± one standard deviation) decayed, missing, or filled surfaces and the CS children had 8.00 ± 2.77 (mean ± one standard deviation) decayed, missing, or filled surfaces, which was statistically significant ($p < 0.01$). The magnitude of the difference between the CR and the CS individuals DMFS scores is shown in Figure 4. A significant difference ($p = 0.002$) in sexual distribution was shown between the CR (m = 18; f = 3) and the CS (m = 8; f = 12) individuals in Figure 5. Racial distribution was found to be similar ($p = 1.00$) between the CR (w = 15; b = 6) and the CS (w = 14; b = 6) subjects as shown in Figure 6.

Quantitation of *S. mutans*

Colonial morphology was used to determine the numbers of total Streptococcal species in the whole saliva of each patient as well as the total numbers of *S. mutans* in each patient's whole saliva. The total oral Streptococcal numbers, the *S. mutans* numbers, and the percentage of *S. mutans* per total oral streptococci of each CR subject are shown in Table II. The Streptococcal numbers from colonial morphology for the CS group are listed in Table III and the percentage of *S. mutans*/total oral streptococci are

compared between the CR and CS individuals in Figure 7. Included in the table are the total *S. mutans* numbers, the total Streptococcal numbers in each subject's whole saliva, and the percentage of *S. mutans* per total oral streptococci for each subject.

It was found that the salivary *S. mutans* total numbers were significantly higher ($p < 0.05$) in the CS individuals as compared to the CR individuals. The average percentage of *S. mutans* per total oral streptococci was 31.2 percent for the CS individuals and 1.6 percent for the CR individuals.

Determination of immunoglobulin concentrations and antibody levels

The mean absorbances for the total IgA concentrations from the CR and CS individuals are listed in Table IV and V, respectively. The results indicate that the total parotid salivary IgA concentrations for the CS and CR individuals were not significantly different ($p = 0.50$). The same was true for the comparison between the total whole salivary IgA1 ($p = 0.20$) and IgA2 ($p = 0.27$) concentrations. However, the difference between the total whole salivary IgA concentrations of the CS and CR groups was significant

($p = 0.08$). The comparison of total IgA concentrations between CR and CS subjects is shown in Figure 9.

At this point, the absorbance values for total IgA levels were converted into concentrations (μg/ml equivalents). This was performed by using a purified secretory IgA human colostrum standard to create a standard curve using linear regression. The equation $y = A + B * x$ was used to plot a linear curve with a correlation coefficient of .918. The known concentrations of total salivary IgA standards were plotted on the x axis, and the mean absorbance of each concentration of total salivary IgA standards were plotted on the y axis. This linear regression was used to calculate the unknown parotid and whole total salivary IgA concentrations but could not be used to calculate the total salivary IgA1 or IgA2 concentrations. There are no known well-characterized IgA1 or IgA2 standards. The total parotid and whole salivary IgA concentrations in μg /ml are listed for the CR and CS subjects in Tables VIII and IX, respectively.

The mean salivary IgA antibody levels to *S. mutans* for each of the CR and CS subjects are presented in Tables VI and VII, respectively. Included are the levels of parotid IgA antibody to *S.*

mutans, levels of whole salivary IgA antibody to *S. mutans*, the levels of whole salivary IgA1 antibody to *S. mutans*, and the levels of whole salivary IgA2 antibody to *S. mutans*. No significant differences were found between the CS and CR individuals when comparing the levels of parotid salivary IgA antibody to *S. mutans* ($p = 0.43$), the levels of whole salivary IgA1 antibody to *S. mutans* ($p = 0.19$), or the levels of whole salivary IgA2 antibody to *S. mutans* ($p = 0.93$). However, there was a significant difference noted between the CS and CR individual levels of whole salivary IgA antibody to *S. mutans* ($p = 0.05$). Figure 8 shows a comparison of the levels of IgA antibodies to *S. mutans* between CR and CS subjects.

In order to make a valid comparison between subject samples, proportions of antibody activity to the total immunoglobulin concentrations were calculated: (1) the proportion of parotid salivary IgA antibody to *S. mutans*/total parotid salivary IgA; (2) the proportion of whole salivary IgA antibody to *S. mutans* /total whole salivary IgA; (3) the proportion of whole salivary IgA1 antibody to *S. mutans*/total whole salivary IgA; (4) the proportion of whole salivary IgA1 antibody to *S. mutans*/total whole salivary IgA1; (5) the proportion of whole salivary IgA2 antibody to *S.*

mutans/total whole salivary IgA; and (6) the proportion of IgA2 antibody to *S. mutans*/total whole salivary IgA2. Student's t test was used to calculate p values for each of the six proportions to compare the differences between the CR and CS individuals.

For each individual parotid sample, the proportion of parotid salivary IgA antibody to *S. mutans* levels/parotid total salivary IgA concentration was calculated by dividing the ELISA absorbance of each parotid salivary IgA antibody to *S. mutans* assay by the respective concentration of parotid salivary IgA (μg/ml) for that given sample. Although the CR group proportions were slightly higher than the CS group, the difference was not significant ($p = .26$) as shown in Figure 10.

The proportions of the levels of whole salivary IgA antibody to *S. mutans*/whole total salivary IgA concentration in the whole salivary samples was calculated by dividing the whole salivary IgA antibody to *S. mutans* absorbance by the concentraion of whole total salivary IgA (μg/ml) for that given sample. It was found that the CR group proportions were significantly higher ($p = .06$) than the CS group proportions. The significance of this proportional comparison is shown in Figure 10.

The proportions of the levels whole salivary IgA1 antibody to *S. mutans*/whole salivary total IgA concentrations were calculated the same as above. Although the CR group proportions were higher than the CS group proportions, the difference was not significant ($p = .83$) as shown in Figure 11.

The proportions of the levels of whole salivary IgA1 antibody to *S. mutans*/whole salivary total IgA1 concentrations was calculated the same as above. The CR group proportions were higher in comparison to the CS group proportions, but the difference was not significant ($p = 0.82$) as shown in Figure 12.

The proportions of the levels of whole salivary IgA2 antibody to *S. mutans*/whole salivary total IgA concentrations were calculated the same as above. The CR and the CS group proportions showed relatively equal levels of antibodies in comparison ($p = 0.75$) as shown in Figure 11.

The proportions of the levels of whole salivary IgA2 antibody to *S. mutans*/whole salivary total IgA2 concentrations were calculated the same as above. Although the CS individuals showed a slightly greater proportion, it was found that the CR and the CS group proportions were not significantly different ($p = 0.38$) as

shown in Figure 12. The data for the CR group proportions are reported in Tables X and XI, and the results for the CS group proportions are found in Tables XII and XIII.

It should be noted that during statistical analysis any sample that was found to be outside 2 standard deviations after a p value was calculated, was removed from the sample field and the p value was recalculated. A summary of the t test statistics including the CR and CS mean values, corresponding standard deviations and p values are listed in Tables XIV and XV.

TABLES AND FIGURES

TABLE I

Population characteristics of caries-resistant and caries-susceptible subjects

	Total Population [a]	Caries-Susceptible Population [b]	Caries-Resistant Population [c]
Age (mean) years	9.62±1.40 [d]	9.46±1.39	9.78±1.41
Sex: # of Females	15	12	3
Sex: # of Males	26	8	18
Race: # of Whites	29	14	15
Race: # of Blacks	12	6	6

[a] Total study population of 41 subjects.
[b] Caries-susceptible population of 20 subjects.
[c] Caries-resistant population of 21 subjects.
[d] Mean age in years ± one standard deviation.

TABLE II

Numbers of *S. mutans* and oral Streptococcal species from caries-resistant subjects

Group and Subject #	Mean Total Strep [a]	Mean Total *S. mutans* [b]	% *S. mutans* of Total Strep [c]
CR-1	3.13E+05 [d]	0E+00	0.00%
CR-2	5.95E+06	9.76E+03	0.16%
CR-3	3.61E+06	2.44E+03	0.07%
CR-4	1.44E+07	2.36E+05	1.64%
CR-5	5.22E+06	8.54E+03	0.16%
CR-6	1.12E+07	4.05E+02	0.40%
CR-7	1.72E+07	2.17E+07	100.00%
CR-8	1.53E+07	6.40E+05	0.42%
CR-9	1.57E+07	7.06E+04	0.45%
CR-10	2.30E+07	2.15E+05	0.94%
CR-11	2.20E+06	0E+00	0.00%
CR-12	1.59E+07	5.13E+04	0.32%
CR-13	2.22E+07	5.45E+05	2.45%
CR-14	1.71E+07	6.61E+04	0.39%
CR-15	1.85E+06	4.15E+05	22.40%
CR-16	1.70E+07	0E+00	0.00%
CR-17	4.60E+06	0E+00	0.00%
CR-18	1.74E+07	6.71E+04	0.39%
CR-19	1.26E+07	2.82E+05	2.23%
CR-20	3.01E+06	0E+00	0.00%
CR-21	1.99E+07	1.02E+03	0.01%
CR Mean [e]	1.17E+07 ± 7.29E+06	1.16E+06 ± 4.71E+06	1.60% [f] ± 4.83%

[a] The mean total Streptococcal count found in each ml of whole saliva.

[b] The mean total *S. mutans* count found in each ml of whole saliva.

[c] Total *S. mutans* count divided by the total Streptococcal count multiplied by 100 percent.

[d] Scientific notation expressed as the decimal number followed by the exponentiation symbol E and an integer in the form *+ii* for the exponent.

[e] The total mean values for the caries resistant group ± one standard deviation.

[f] Significantly different ($p = 0.01$) than CS group.

TABLE III

Numbers of *S. mutans* and oral Streptococcal species from caries-susceptible subjects

Group and Subject #	Mean Total Strep [a]	Mean Total *S. mutans* [b]	% *S. mutans* of Total Strep [c]
CS-301	7.13E+06 [d]	6.91E+04	0.97%
CS-302	1.07E+06	1.44E+07	100.00%
CS-303	2.48E+07	7.92E+06	31.93%
CS-304	2.51E+07	1.06E+06	4.23%
CS-305	1.01E+07	1.88E+07	100.00%
CS-306	1.71E+07	3.13E+05	1.83%
CS-307	1.42E+07	6.26E+04	4.41%
CS-308	5.15E+06	3.46E+05	6.71%
CS-309	1.78E+07	2.05E+05	1.15%
CS-310	6.39E+06	1.79E+05	2.80%
CS-311	4.09E+06	7.11E+04	1.74%
CS-312	1.92E+06	1.52E+06	79.09%
CS-313	8.03E+06	1.26E+05	1.57%
CS-314	2.11E+07	2.09E+07	99.20%
CS-315	TNTC [e]	TNTC	ND [f]
CS-316	1.58E+07	8.72E+06	55.21%
CS-317	7.23E+06	5.37E+05	7.42%
CS-318	TNTC	TNTC	ND
CS-319	TNTC	TNTC	ND
CS-320	1.17E+07	3.75E+06	32.05%
CS Mean [g]	1.17E+07 ± 7.60E+06	4.65E+06 ± 7.02E+06	31.19% [h] ± 39.37%

[a] The mean total Streptococcal count found in each ml of whole saliva.

[b] The mean total *S. mutans* ocunt found in each ml of whole saliva.

[c] The total *S. mutans* count was divided by the total Streptococcal count and multiplied by 100 percent.

[d] Scientific notation expressed as the decimal number followed by the exponentiation symbol E and an integer in the form *+ii* for the exponent.

[e] Too numerous to count (> 10E+08).

[f] Not determined

[g] The total mean values for the caries susceptible group ± one standard deviation.

[h] Significantly different ($p = 0.01$) than CR group.

TABLE IV

Levels of total IgA, IgA1 and IgA2 in parotid and whole saliva from caries-resistant subjects

Group and Subject #	Parotid saliva (IgA)	Whole saliva (IgA)	Whole saliva (IgA1)	Whole saliva (IgA2)
CR-1	1.379 [a]	0.842	0.246	0.196
CR-2	0.057	0.771	0.261	0.226
CR-3	1.353	0.834	0.232	0.194
CR-4	1.445	0.610	0.141	0.145
CR-5	1.359	0.750	0.141	0.180
CR-6	1.253	0.913	0.249	0.232
CR-7	1.261	0.907	0.278	0.256
CR-8	1.437	0.935	0.290	0.227
CR-9	1.354	1.097	0.329	0.219
CR-10	1.788	0.990	0.489	0.182
CR-11	0.174	0.787	0.398	0.101
CR-12	1.708	0.958	0.618	0.133
CR-13	1.640	0.943	0.510	0.139
CR-14	1.765	0.880	0.404	0.125
CR-15	1.921	0.922	0.527	0.181
CR-16	1.539	0.790	0.229	0.110
CR-17	1.667	0.753	0.194	0.128
CR-18	1.902	0.980	0.274	0.160
CR-19	2.377	1.045	0.262	0.290
CR-20	2.154	1.094	0.307	0.288
CR-21	2.505	1.114	0.287	0.270
CR Mean [b]	1.670	0.910	0.300	0.190
	±0.370	±0.120	±0.110	±0.060

[a] Mean ELISA absorbance at a wavelength of 490 nm.

[b] The total mean values ± SD for the caries-resistant group.

TABLE V

Levels of total IgA, IgA1 and IgA2 in parotid and whole saliva from caries-susceptible subjects

Group and Subject #	Parotid saliva (IgA)	Whole saliva (IgA)	Whole saliva (IgA1)	Whole saliva (IgA2)
CS-301	1.695 [a]	0.884	0.266	0.252
CS-302	1.621	0.747	0.147	0.161
CS-303	1.548	1.064	0.256	0.217
CS-304	1.628	0.834	0.203	0.237
CS-305	1.579	0.912	0.246	0.215
CS-306	1.949	0.809	0.159	0.175
CS-307	1.545	0.890	0.213	0.174
CS-308	1.493	0.899	0.195	0.203
CS-309	1.486	1.006	0.262	0.229
CS-310	1.662	0.872	0.383	0.163
CS-311	1.283	0.872	0.453	0.154
CS-312	1.619	0.669	0.275	0.079
CS-313	1.882	0.829	0.480	0.105
CS-314	1.094	0.749	0.260	0.089
CS-315	1.384	0.917	0.350	0.152
CS-316	1.436	0.967	0.309	0.125
CS-317	1.755	0.970	0.303	0.160
CS-318	1.866	0.675	0.135	0.099
CS-319	2.005	0.752	0.228	0.174
CS-320	1.066	0.947	0.327	0.240
CS Mean [b]	1.610	0.850	0.260	0.170
	±0.230	±0.100	±0.080	±0.050

[a] Mean ELISA absorbance at wavelength of 490 nm.

[b] The total mean values ± SD for the caries-susceptible group.

TABLE VI

Salivary IgA antibody levels to *S. mutans* from caries-resistant subjects

Group and Subject #	Parotid saliva (IgA)	Whole saliva (IgA)	Whole saliva (IgA1)	Whole saliva (IgA2)
CR-1	0.261 [a]	0.839	0.245	0.531
CR-2	- [b]	0.501	0.169	0.386
CR-3	0.256	0.124	0.497	0.193
CR-4	0.458	0.392	0.531	0.252
CR-5	0.189	0.481	0.245	0.236
CR-6	0.387	0.684	0.440	0.345
CR-7	0.395	8.440	0.271	0.475
CR-8	0.205	1.040	0.211	0.305
CR-9	0.227	1.790	0.450	0.523
CR-10	0.207	0.910	0.532	0.247
CR-11	0.146	0.234	0.355	0.187
CR-12	0.221	0.935	0.724	0.441
CR-13	0.343	0.529	0.508	0.480
CR-14	0.243	0.784	0.453	0.225
CR-15	0.482	0.640	0.245	0.311
CR-16	0.279	0.311	0.353	0.255
CR-17	0.303	0.316	0.282	0.243
CR-18	0.492	2.200	0.389	0.375
CR-19	0.681	2.070	0.655	0.517
CR-20	0.294	0.443	0.277	0.286
CR-21	0.759	1.950	0.407	0.456
CR Mean [c]	0.341	0.810	0.350	0.346
	±0.163	±0.570	±0.130	±0.120

[a] Mean ELISA absorbance at a wavelength of 490 nm.

[b] Insufficient volume of saliva.

[c] The total mean values ± SD for the caries-resistant group.

TABLE VII

Salivary IgA antibody levels to *S. mutans* from caries-susceptible subjects

Group and Subject #	Parotid saliva (IgA)	Whole saliva (IgA)	Whole saliva (IgA1)	Whole saliva (IgA2)
CS-301	0.325 [a]	0.474	0.390	0.382
CS-302	0.199	0.367	0.247	0.242
CS-303	0.268	2.520	0.709	0.554
CS-304	0.407	1.390	0.571	0.472
CS-305	0.284	0.622	0.343	0.357
CS-306	0.190	0.303	0.306	0.272
CS-307	0.238	0.974	0.078	0.439
CS-308	0.333	0.874	0.340	0.373
CS-309	0.393	0.462	0.373	0.342
CS-310	0.770	0.283	0.291	0.250
CS-311	0.455	0.648	0.258	0.234
CS-312	0.213	0.176	0.181	0.153
CS-313	0.900	0.743	0.564	0.566
CS-314	0.233	0.283	0.199	0.182
CS-315	0.177	0.183	0.263	0.220
CS-316	0.155	0.258	0.286	0.325
CS-317	0.291	0.222	0.688	0.594
CS-318	0.275	0.279	0.242	0.203
CS-319	0.357	0.241	0.257	0.213
CS-320	0.287	3.010	0.376	0.627
CS Mean [b]	0.338 ±0.189	0.490 ±0.330	0.320 ±0.110	0.350 ±0.150

[a] Mean ELISA absorbance at a wavelength of 490nm.

[b] The total mean values ± SD for the caries-susceptible group.

TABLE VIII

Concentrations of total salivary IgA from caries-resistant subjects converted using secretory IgA human colostrum standards

Group and Subject #	Parotid Saliva (IgA)	Whole Saliva (IgA)
CR-1	56.61 [a]	24.03
CR-2	- [b]	19.73
CR-3	55.04	23.55
CR-4	60.62	9.96
CR-5	55.40	18.45
CR-6	48.97	28.34
CR-7	49.45	27.98
CR-8	60.13	29.68
CR-9	55.10	39.50
CR-10	81.43	33.01
CR-11	0.00	20.70
CR-12	76.57	31.07
CR-13	72.45	30.16
CR-14	80.03	26.34
CR-15	89.50	28.89
CR-16	66.32	20.88
CR-17	74.09	18.63
CR-18	88.34	32.41
CR-19	117.16	36.35
CR-20	103.63	39.32
CR-21	124.93	40.54
CR Mean [c]	74.51 ±22.24	27.60 ±7.94

[a] Total salivary IgA concentrations (μg/ml).

[b] Insufficient sample

[c] The total mean values ± SD for the caries-resistant group.

TABLE IX

Concentrations of total salivary IgA from caries-susceptible subjects converted using secretory IgA human colostrum standard

Group and Subject #	Parotid Saliva (IgA)	Whole Saliva (IgA)
CS-301	75.78 [a]	26.58
CS-302	71.29	18.27
CS-303	66.87	37.50
CS-304	71.72	23.55
CS-305	68.75	28.28
CS-306	91.19	22.03
CS-307	66.68	26.95
CS-308	63.53	27.49
CS-309	63.10	33.98
CS-310	73.78	25.85
CS-311	50.79	25.85
CS-312	71.17	13.54
CS-313	87.13	23.25
CS-314	39.32	18.39
CS-315	56.92	28.58
CS-316	60.07	31.62
CS-317	79.42	31.80
CS-318	86.16	13.90
CS-319	94.59	18.57
CS-320	37.62	30.40
CS Mean [b]	68.79 ± 15.32	25.32 ± 6.45

[a] Total salivary IgA concentrations (μg/ml).

[b] The total mean values ± SD for the caries-susceptible group.

TABLE X

Proportion of salivary IgA antibody to *S. mutans*/ total IgA levels from caries-resistant subjects

Subject	Parotid saliva (IgA / IgA)	Whole saliva (IgA / IgA)	Whole saliva (IgA1 / IgA)
CR-1	0.005 [a]	0.035	0.021
CR-2	- [b]	0.025	0.023
CR-3	0.005	0.005	0.010
CR-4	0.008	0.039	0.035
CR-5	0.008	0.026	0.015
CR-6	0.003	0.024	0.014
CR-7	0.008	0.602	0.023
CR-8	0.003	0.035	0.009
CR-9	0.004	0.045	0.010
CR-10	0.003	0.028	0.007
CR-11	0.003	0.011	0.008
CR-12	0.005	0.030	0.016
CR-13	0.003	0.018	0.018
CR-14	0.005	0.030	0.009
CR-15	0.004	0.022	0.015
CR-16	0.004	0.015	0.013
CR-17	0.006	0.017	0.011
CR-18	0.006	0.068	0.014
CR-19	0.003	0.029	0.015
CR-20	0.006	0.011	0.009
CR-21	0.004	0.048	0.018
CR Mean [c]	0.005 [d] ±0.005	0.028 [e] ±0.020	0.014 [f] ±0.010

[a] Mean ELISA absorbance of IgA to *S. mutans* divided by the mean total IgA concentration.

[b] Insufficient sample.

[c] The total mean values ± SD for the caries-resistant group.

[d] Comparison to caries susceptible; ($p = 0.73$).

[e] Comparison to caries susceptible; ($p = 0.06$).

[f] Comparison to caries susceptible; ($p = 0.83$).

TABLE XI

Proportion of salivary IgA antibody to *S. mutans*/ total IgA levels from caries-resistant subjects

Group and Subject #	Whole saliva (IgA1 / IgA1)	Whole saliva (IgA2 / IgA)	Whole saliva (IgA2 / IgA2)
CR-1	2.065 [a]	0.022	2.709
CR-2	1.736	0.020	1.708
CR-3	1.056	0.008	0.995
CR-4	2.504	0.025	1.738
CR-5	2.000	0.013	1.311
CR-6	1.562	0.012	1.487
CR-7	2.356	0.017	1.855
CR-8	0.955	0.010	1.344
CR-9	1.237	0.013	2.388
CR-10	0.501	0.007	1.357
CR-11	0.425	0.009	1.851
CR-12	0.804	0.014	3.316
CR-13	1.041	0.016	3.453
CR-14	0.606	0.009	1.800
CR-15	0.835	0.011	1.718
CR-16	1.183	0.012	2.318
CR-17	1.088	0.013	1.898
CR-18	1.640	0.023	2.344
CR-19	2.031	0.014	1.783
CR-20	1.156	0.007	0.993
CR-21	2.523	0.011	1.689
CR Mean [b]	1.430 [c] ±0.660	0.013 [d] ±0.010	1.910 [e] ±0.660

[a] Mean ELISA absorbance of IgA to *S. mutans* divided by the mean total IgA concentration.

[b] The total mean values ± SD for the caries-resistant group.

[c] Comparison to caries susceptible; ($p = 0.82$).

[d] Comparison to caries susceptible; ($p = 0.75$).

[e] Comparison to caries susceptible; ($p = 0.38$).

TABLE XII

Proportion of salivary IgA antibody to *S. mutans*/ total IgA levels from caries-susceptible subjects

Group and Subject #	Parotid saliva (IgA / IgA)	Whole saliva (IgA / IgA)	Whole saliva (IgA1 / IgA)
CS-301	0.004 [a]	0.018	0.015
CS-302	0.003	0.020	0.014
CS-303	0.004	0.067	0.019
CS-304	0.006	0.059	0.024
CS-305	0.004	0.022	0.012
CS-306	0.002	0.014	0.014
CS-307	0.004	0.036	0.003
CS-308	0.005	0.032	0.012
CS-309	0.006	0.014	0.011
CS-310	0.010	0.011	0.011
CS-311	0.009	0.025	0.010
CS-312	0.003	0.013	0.013
CS-313	0.010	0.032	0.024
CS-314	0.006	0.015	0.011
CS-315	0.003	0.006	0.009
CS-316	0.003	0.008	0.009
CS-317	0.004	0.007	0.022
CS-318	0.003	0.020	0.017
CS-319	0.004	0.013	0.014
CS-320	0.008	0.099	0.012
CS Mean [b]	0.004 [c] ±0.002	0.020 [d] ±0.010	0.014 [e] ±0.010

[a] Mean ELISA absorbance of IgA to *S. mutans* divided by the mean total IgA concentration.

[b] The total mean values ± SD for the caries-susceptible group.

[c] Comparison to caries resistant; ($p = 0.26$).

[d] Comparison to caries resistant; ($p = 0.06$).

[e] Comparison to caries resistant; ($p = 0.83$).

TABLE XIII

Proportion of salivary IgA antibody to *S. mutans*/ total IgA levels from caries-susceptible subjects

Group and Subject #	Whole saliva (IgA1 / IgA1)	Whole saliva (IgA2 / IgA)	Whole saliva (IgA2 / IgA2)
CS-301	1.466 [a]	0.014	1.516
CS-302	1.680	0.013	1.503
CS-303	2.770	0.015	2.553
CS-304	2.813	0.020	1.992
CS-305	1.394	0.013	1.660
CS-306	1.925	0.010	1.554
CS-307	0.366	0.016	2.523
CS-308	1.744	0.014	1.837
CS-309	1.424	0.010	1.493
CS-310	0.760	0.010	1.534
CS-311	0.570	0.010	1.937
CS-312	0.658	0.011	1.519
CS-313	1.175	0.024	5.390
CS-314	0.765	0.010	2.045
CS-315	0.751	0.008	1.447
CS-316	0.926	0.010	2.600
CS-317	2.271	0.019	3.713
CS-318	1.793	0.015	2.051
CS-319	1.127	0.011	1.224
CS-320	1.150	0.021	2.613
CS Mean [b]	1.390 [c] ±0.710	0.014 [d] ±0.010	2.140 [e] ±0.220

[a] Mean ELISA absorbance of IgA to *S. mutans* divided by the mean total IgA concentration.

[b] The total mean values ± SD for the caries-susceptible group.

[c] Comparison of caries resistant; ($p = 0.82$).

[d] Comparison of caries resistant; ($p = 0.75$).

[e] Comparison of caries resistant; ($p = 0.38$).

TABLE XIV

Summary of t test statistics

Sample	CR Mean	CS Mean	P Value [a]
Parotid IgA Ab to *S. mutans*	0.34 ± 0.16 [b]	0.34 ± 0.19	0.82
Parotid Total IgA	1.67 ± 0.37 [c]	1.61 ± 0.23	0.50
Whole IgA Ab to *S. mutans*	0.81 ± 0.57 [b]	0.49 ± 0.33	0.05
Whole Total IgA	0.91 ± 0.12 [c]	0.85 ± 0.10	0.08
Whole IgA1 Ab to *S. mutans*	0.38 ± 0.13 [b]	0.32 ± 0.11	0.19
Whole Total IgA1	0.30 ± 0.11 [c]	0.26 ± 0.08	0.20
Whole IgA2 Ab to *S. mutans*	0.35 ± 0.12 [b]	0.35 ± 0.15	0.93
Whole Total IgA2	0.19 ± 0.06 [c]	0.17 ± 0.05	0.27

[a] P value calculated using Student's t test.

[b] Mean ELISA absorbance at 490 nm ± standard deviation.

[c] Mean total salivary IgA concentration (μg / ml) ± standard deviation.

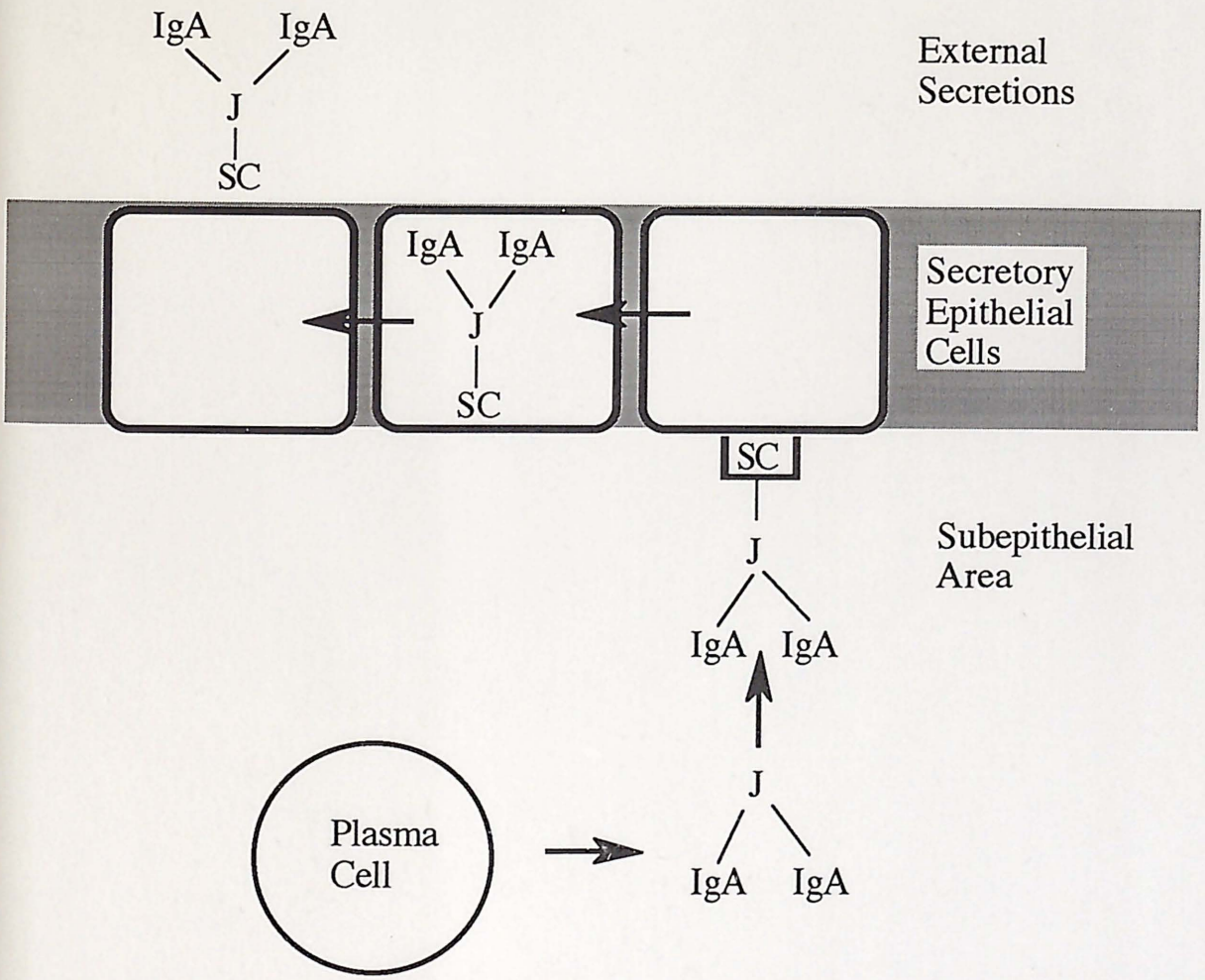
TABLE XV

Summary of t test statistics

Sample	CR Mean	CS Mean	P Value [a]
Parotid IgA Ab to *S. mutans* / Total IgA	0.005 ± 0.002 [b]	0.005 ± 0.002	0.73
Whole IgA Ab to *S. mutans* / Total IgA	0.03 ± 0.02	0.02 ± 0.01	0.06
Whole IgA1 Ab to *S. mutans* / Total IgA	0.01 ± 0.01	0.01 ± 0.01	0.83
Whole IgA1 Ab to *S. mutans* / Total IgA1	1.43 ± 0.66	1.39 ± 0.71	0.82
Whole IgA2 Ab to *S. mutans* / Total IgA	0.01 ± 0.01	0.01 ± 0.01	0.75
Whole IgA2 Ab to *S. mutans* / Total IgA2	1.91 ± 0.66	2.14 ± 0.22	0.38

[a] P value calculated using Student's t test.

[b] Mean ELISA absorbance at 490 nm of IgA antibody to *S. mutans* divided by the mean total salivary IgA concentration (μg/ml).



IgA:J Chain Translocation by Secretory Component

Figure 1. Demonstration of IgA:J chain translocation from subepithelial areas to external secretions by secretory component.

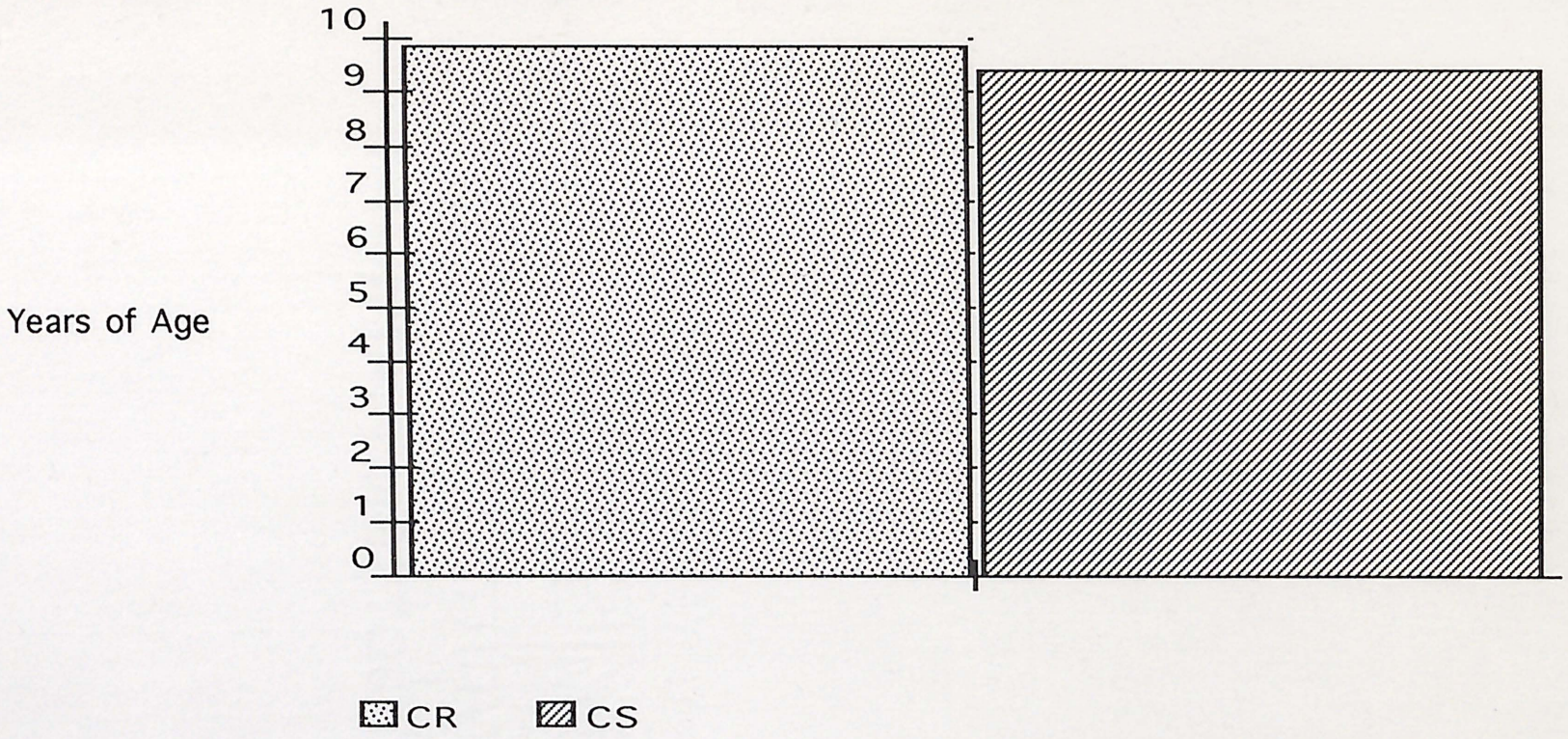


FIGURE 2. Chronological age in years of caries-resistant and caries-susceptible subjects.

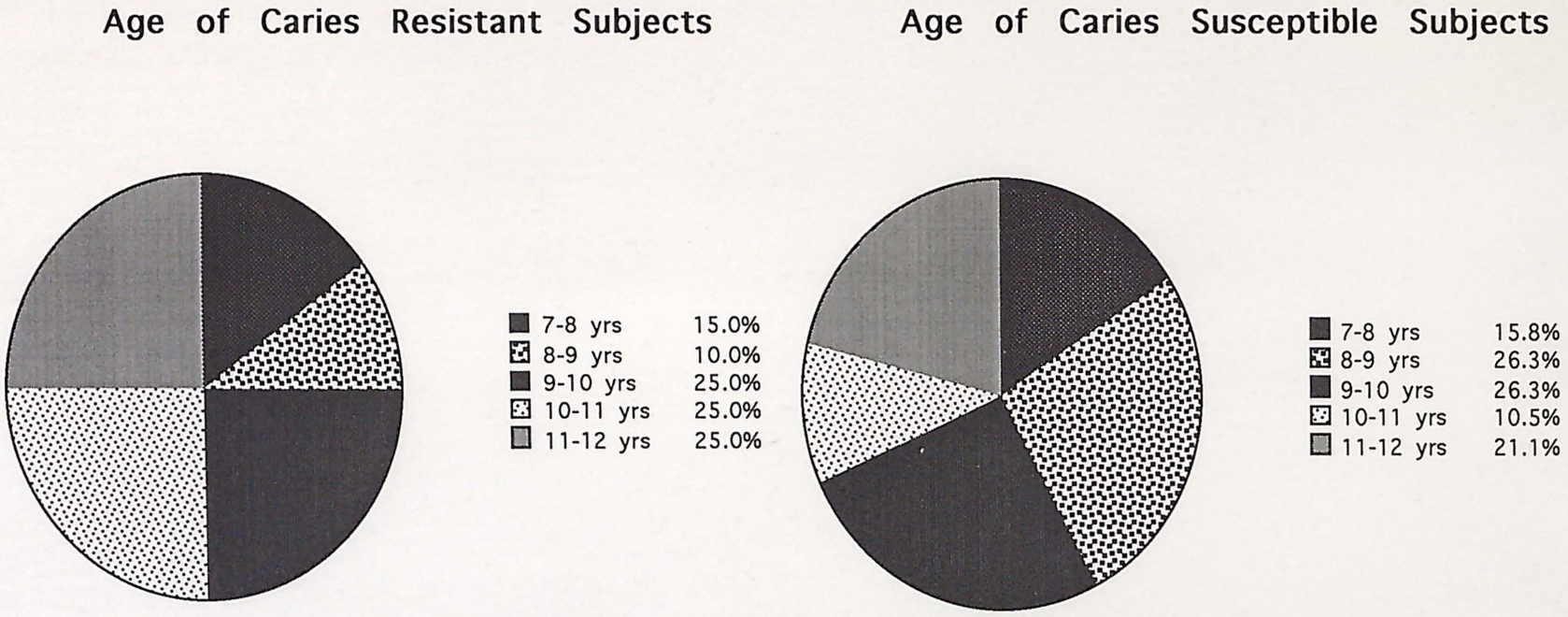


FIGURE 3. Subject distribution of each year of age for the caries-resistant and caries-susceptible subjects.

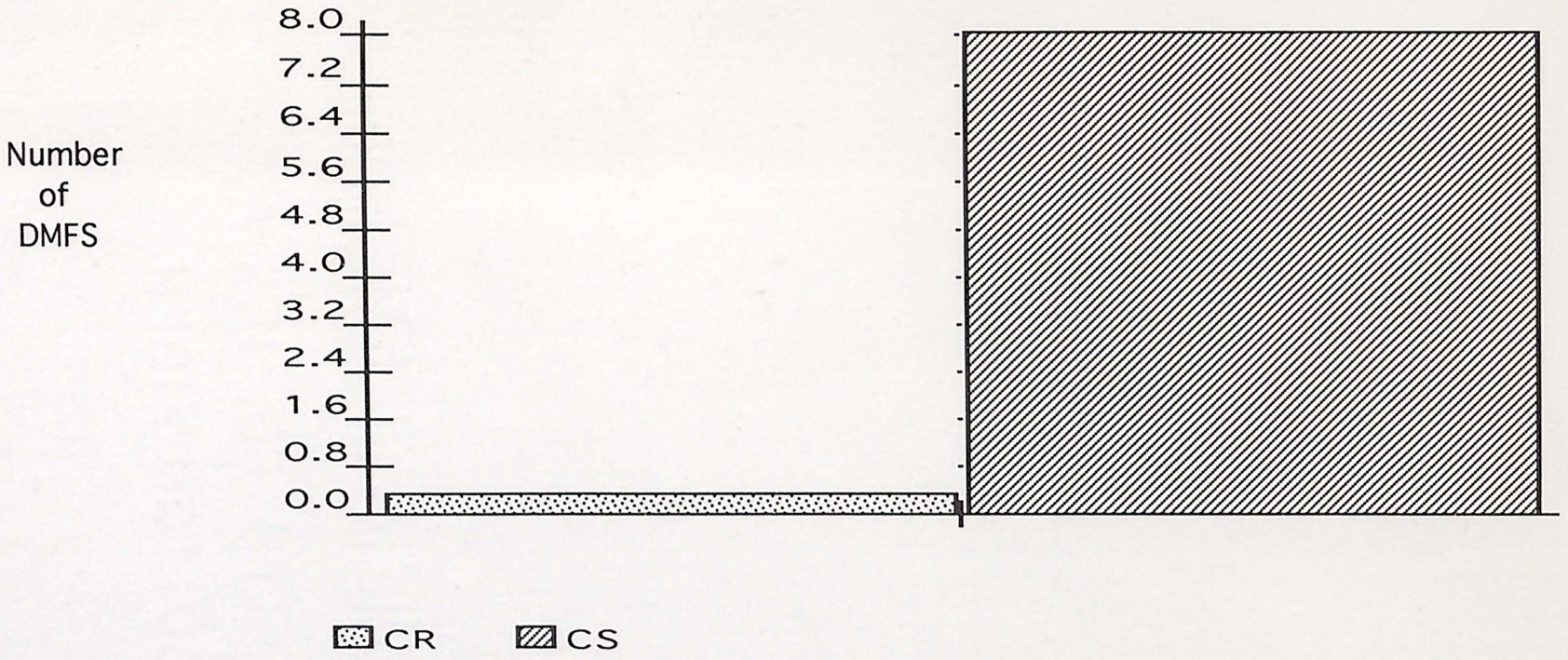


FIGURE 4. Comparison of the number of decayed, missing, or filled tooth surfaces from caries-resistant and caries-susceptible subjects as detected upon oral and radiographic examination.

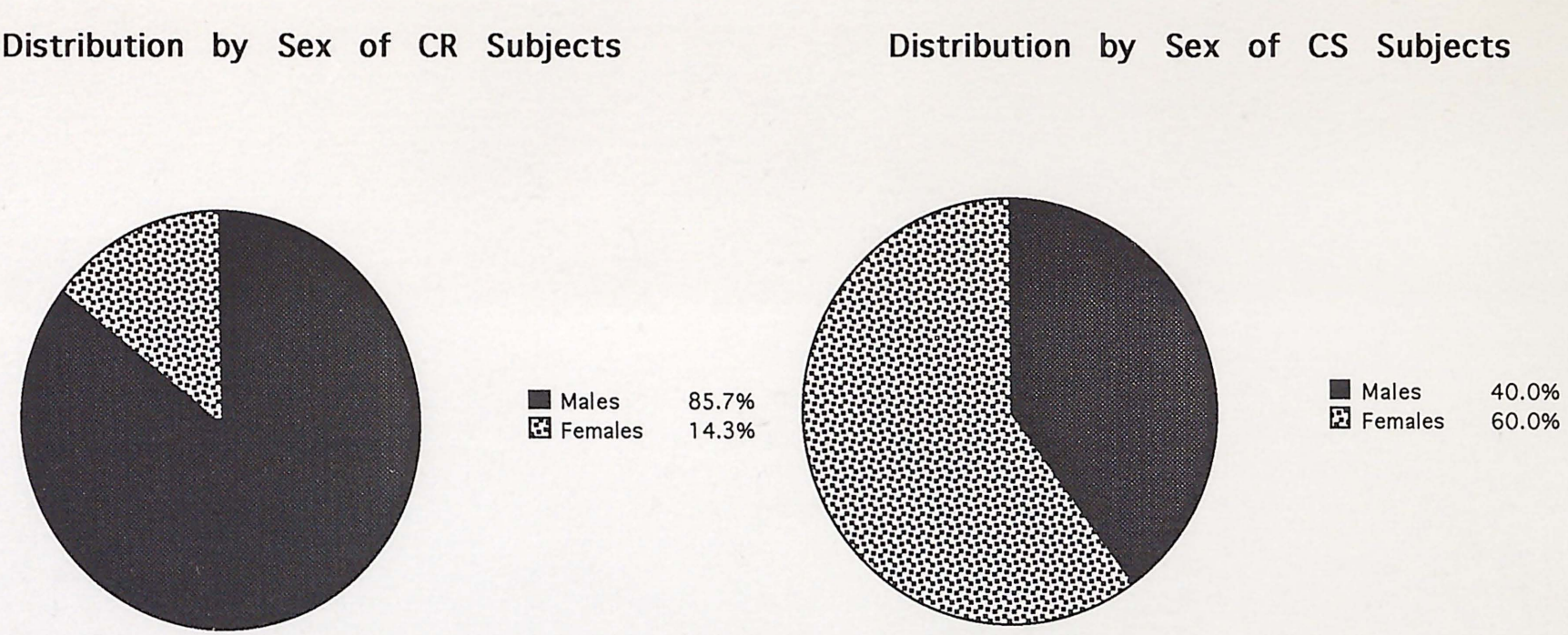


FIGURE 5. Distribution by sex of the caries-resistant and caries-susceptible subjects.

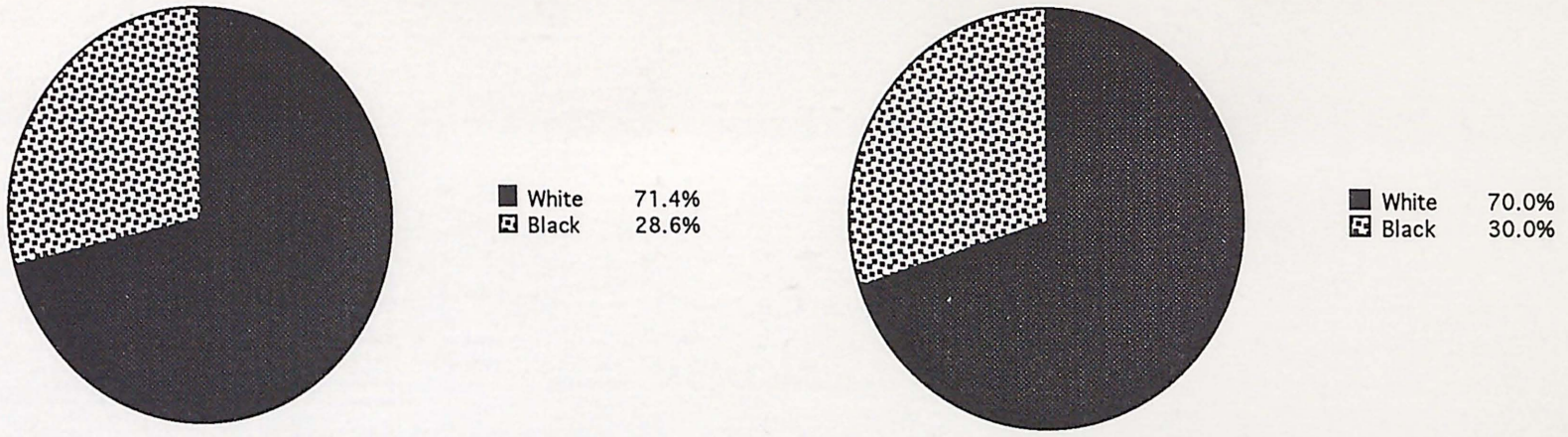


FIGURE 6. Distribution by race of the caries-resistant and caries-susceptible subjects.

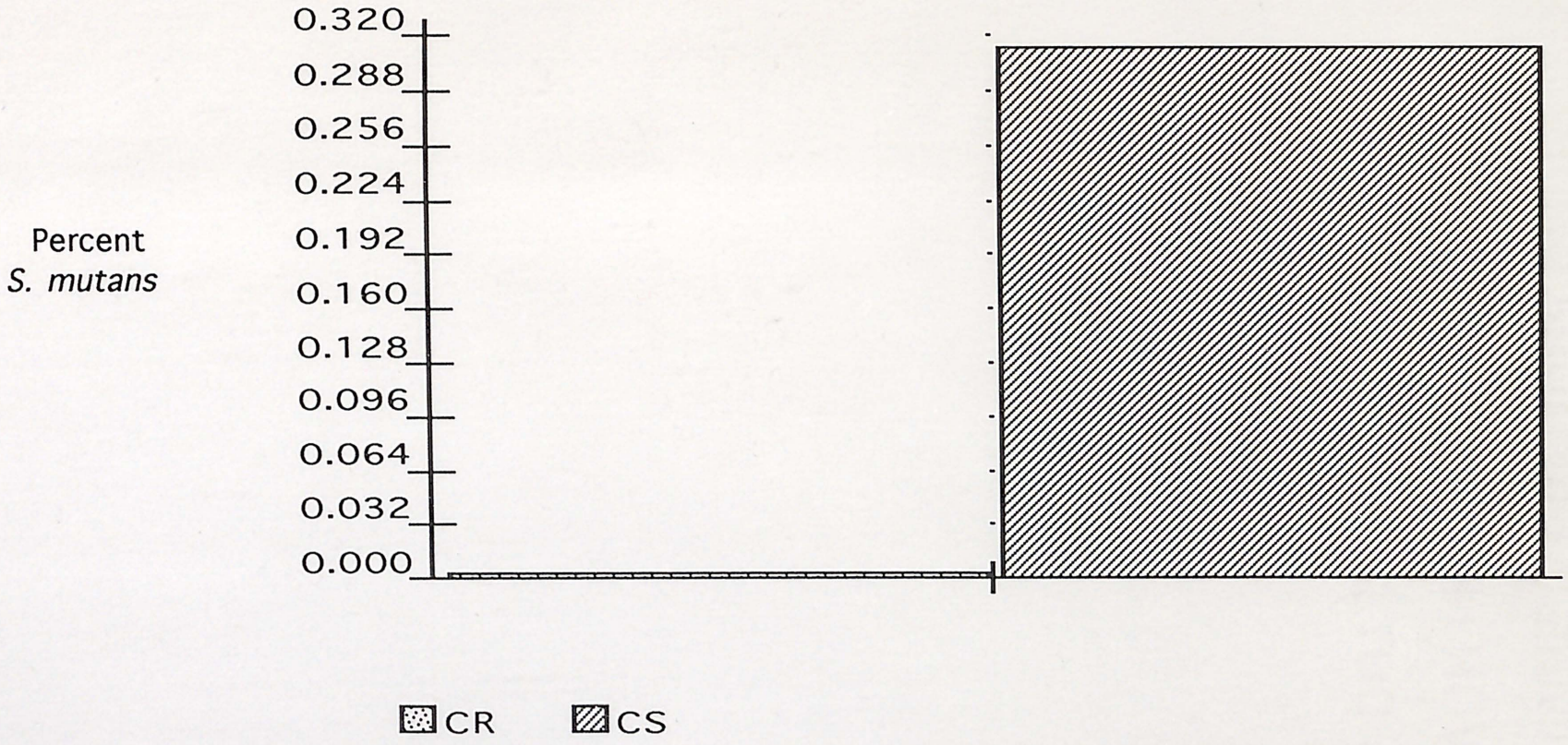


FIGURE 7. Comparison of percentage of *S. mutans*/total oral streptococci from caries-resistant and caries-susceptible subjects determined by colonial morphology from spiral plated cultures.

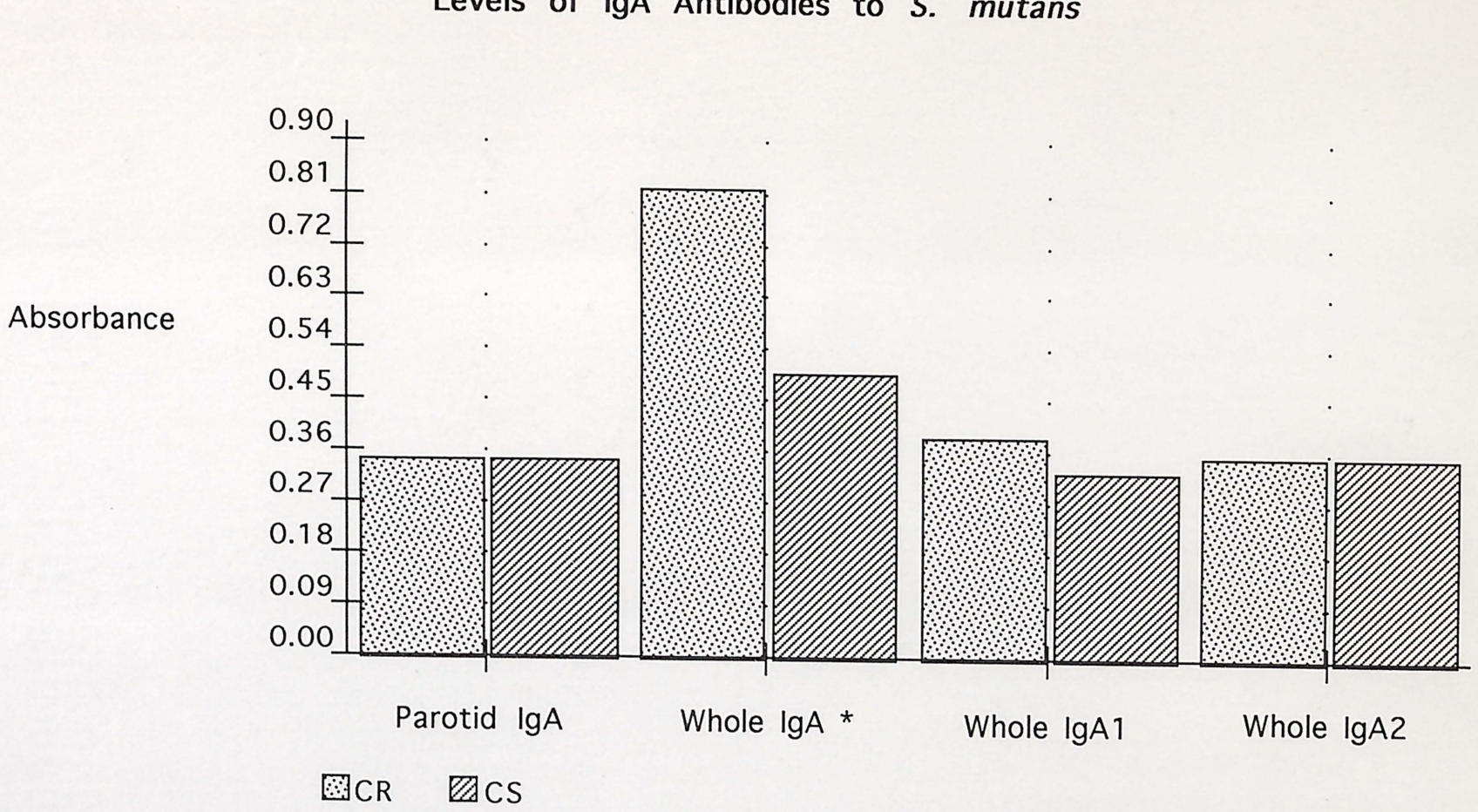


FIGURE 8. Comparison of levels of IgA antibodies to *S. mutans* from caries-resistant and caries-susceptible subjects determined by ELISA. * ($p = 0.05$)

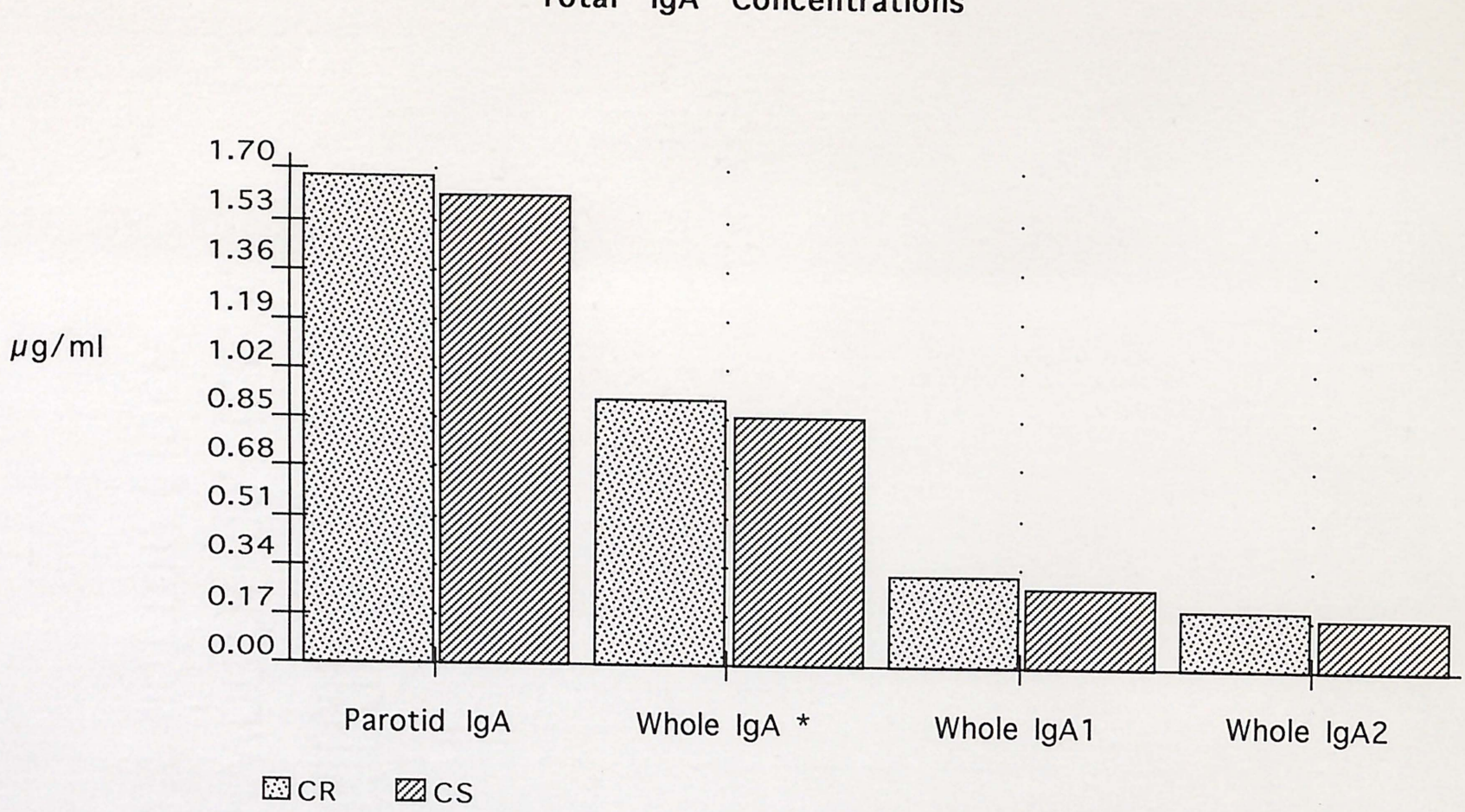


FIGURE 9. Comparison of total IgA concentrations from caries-resistant and caries-susceptible subjects determined by ELISA. * ($p = 0.08$)

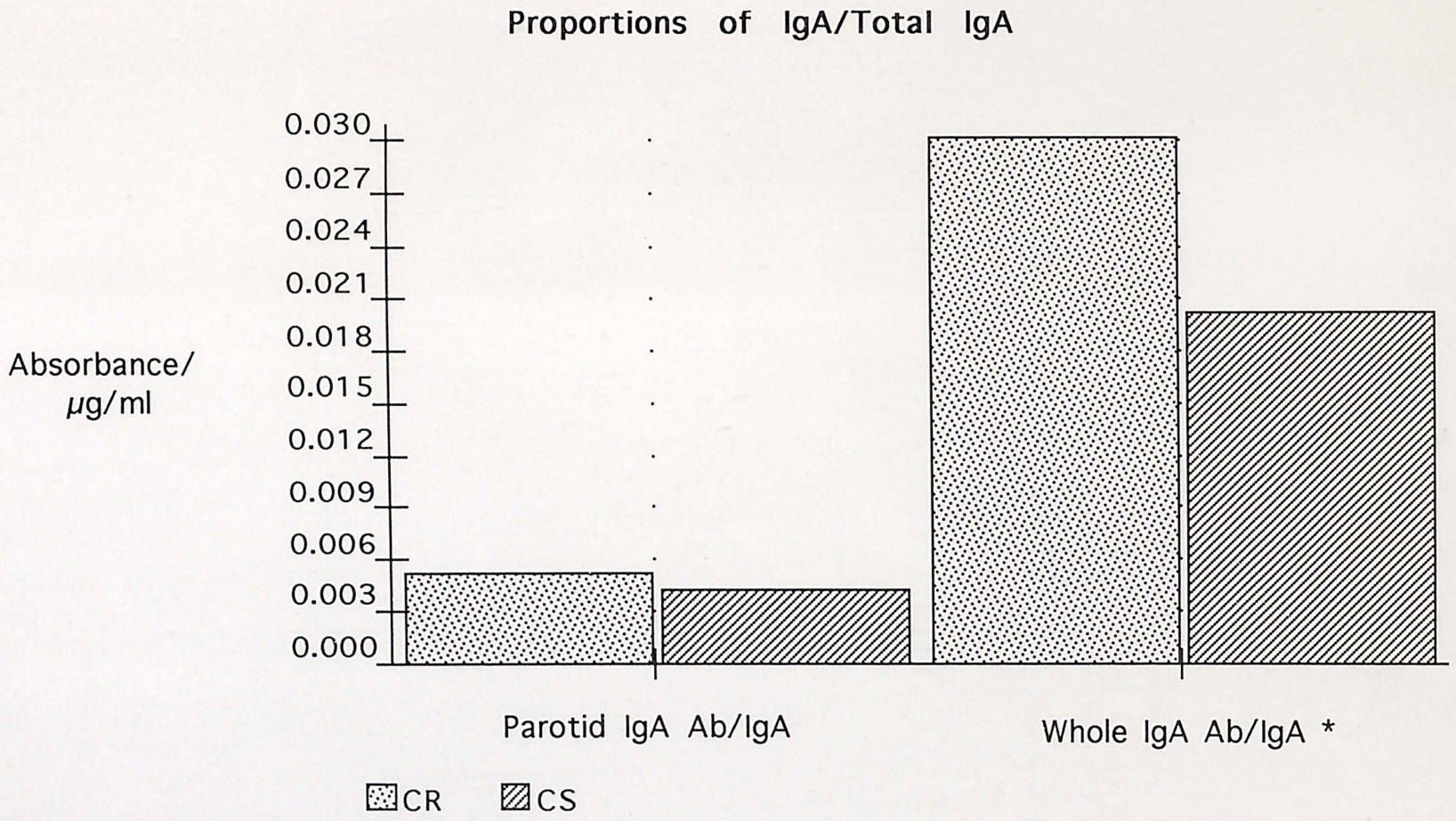


FIGURE 10. Comparison of the proportions of IgA antibody to *S. mutans*/total IgA in parotid saliva or whole saliva from caries-resistant and caries-susceptible subjects determined by ELISA. *(p = 0.06)

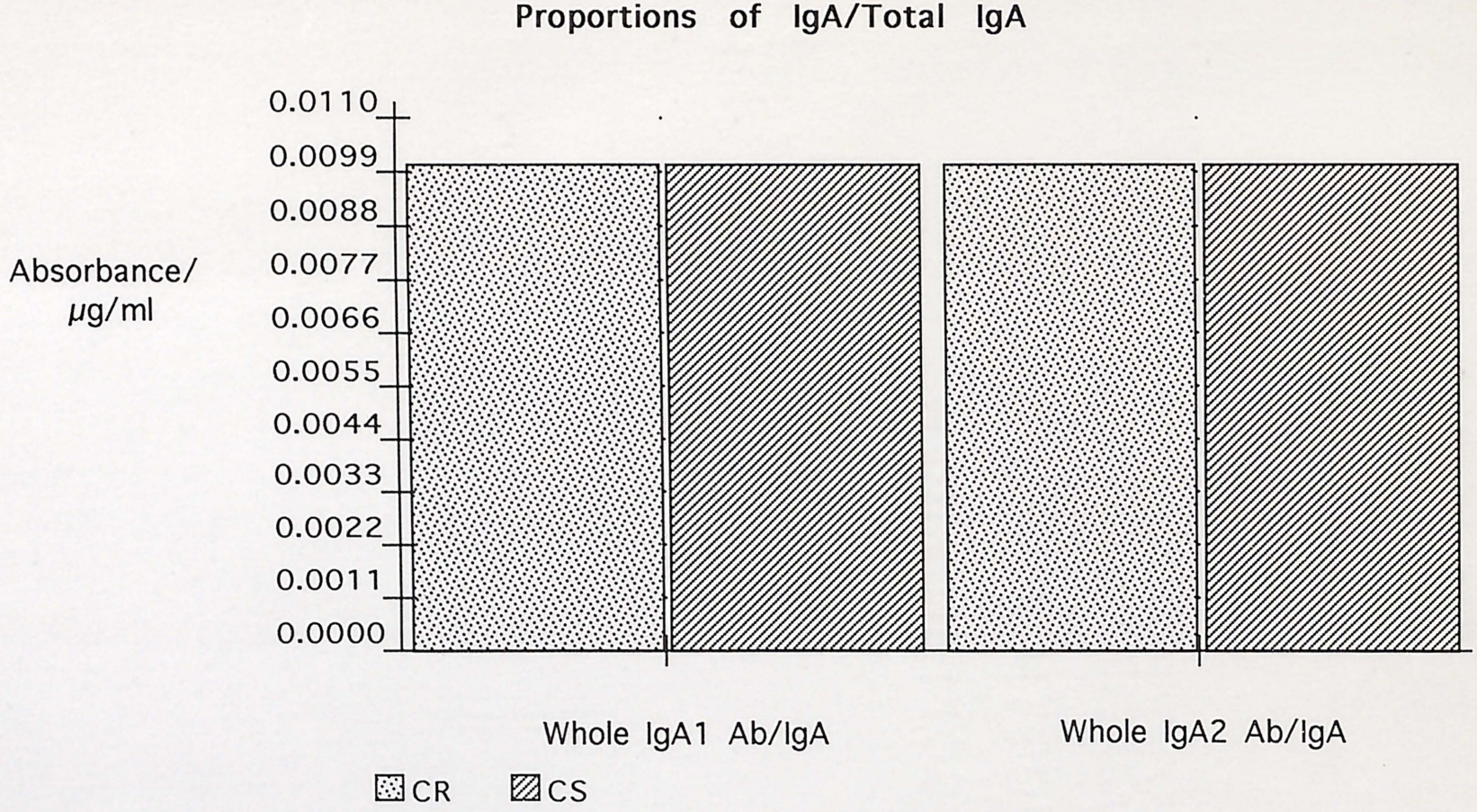


FIGURE 11. Comparison of the proportions of IgA1 and IgA2 antibody to *S. mutans*/total IgA in whole saliva from caries-resistant and caries-susceptible subjects determined by ELISA.

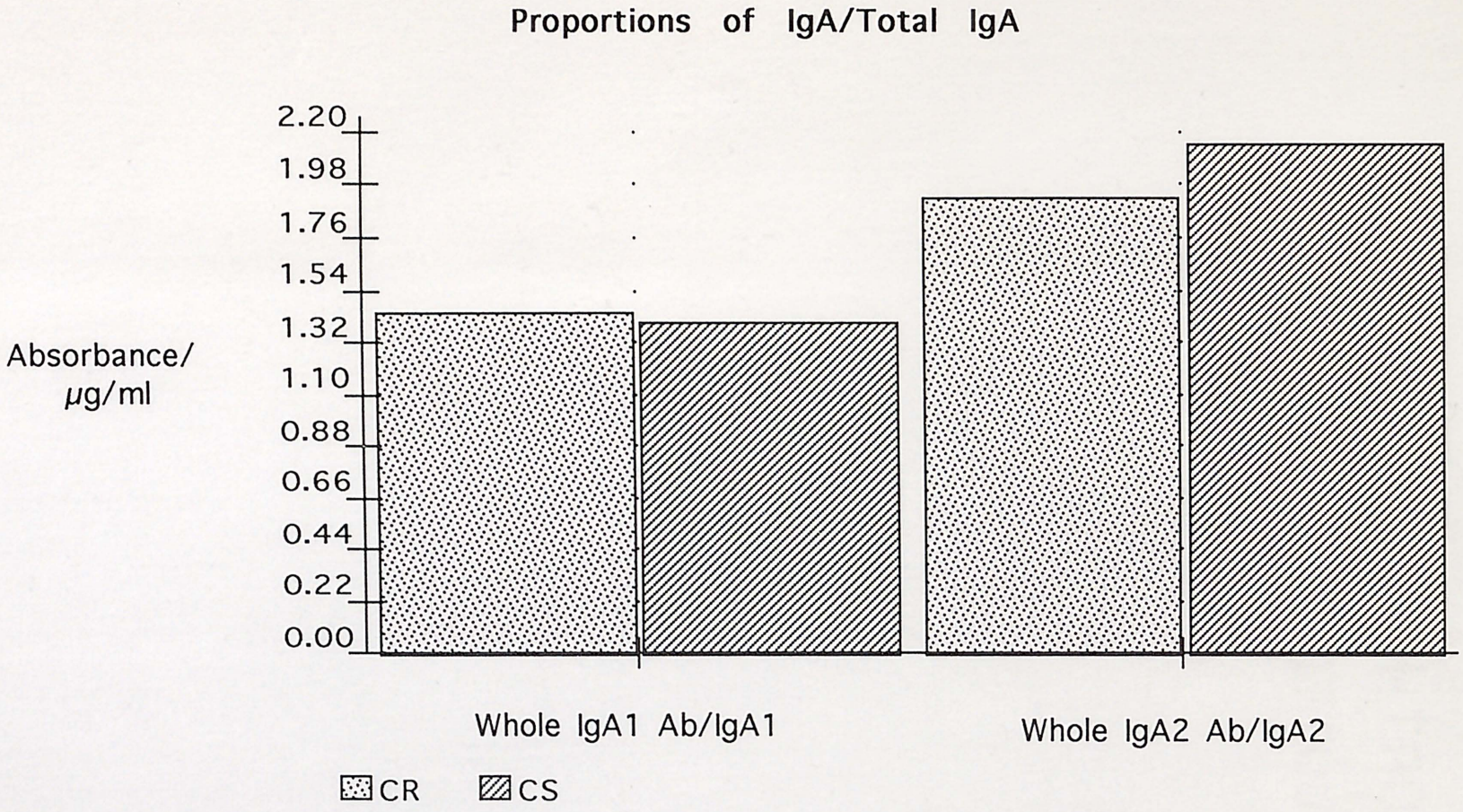


FIGURE 12. Comparison of the proportions of IgA1 antibody to *S. mutans*/total IgA1 and IgA2 to *S. mutans*/total IgA2 in whole saliva from caries-resistant and caries-susceptible subjects determined by ELISA.

DISCUSSION

It has been previously reported by Camling, Gahnberg and Krasse[54] that there is a correlation between the degree of caries activity and total salivary IgA concentrations and also between the DMFS score and total salivary IgA concentrations. The same group also reported that most high-caries subjects had high salivary numbers of *S. mutans* and that any attempts to relate the level of IgA antibodies in whole saliva to the prevalence of caries in an individual should take into consideration the number of salivary *S. mutans*. For these reasons the present study not only examined patients with different levels of caries activity as it related to their salivary IgA concentrations, but also took into consideration what each of their total Streptococcal and *S. mutans* counts were at the time of saliva collection. In this way, it could be shown that not only were the individual caries activity levels compared with the corresponding salivary IgA concentrations but also that each subject had varying numbers of *S. mutans* in their whole saliva.

The present study supports previous investigations that found that subjects with higher caries activity levels had significantly

higher numbers of *S. mutans* in their whole saliva. The study was designed to examine the total Streptococcal and *S. mutans* numbers for each patient and to divide the *S. mutans* numbers by the total oral Streptococcal numbers to calculate the proportion of *S. mutans* for each sample.

Gregory et al.[61,69,70] and other investigators reported a correlation between lower rates of caries activity and higher levels of salivary IgA antibody levels to *S. mutans*. It was also reported that IgA1 and IgA2 antibodies may inhibit *S. mutans* virulence factors and suggest that IgA2 may be more effective than IgA1 at this function. The current study supported findings from these studies in that the levels of whole salivary IgA antibody to *S. mutans* were higher in caries-resistant subjects than the caries-susceptible subjects.

It was found in this investigation that the total whole salivary IgA concentrations, the levels of whole salivary IgA antibody to *S. mutans*, and the mean proportions of the level of whole salivary IgA antibody to *S. mutans*/total whole salivary IgA concentration were significantly higher in the caries-resistant subjects as compared to the caries-susceptible subjects. The total

parotid salivary IgA concentrations, the levels of parotid salivary IgA antibody to *S. mutans* level and the mean proportions of the level of whole salivary IgA antibody to *S. mutans*/total whole salivary IgA concentrations were found not to be significantly different in the caries-resistant versus caries-susceptible subjects. Also, the total whole salivary IgA1 concentrations, the levels of whole salivary IgA1 antibody to *S. mutans* and the proportions of the level of whole salivary IgA1 antibody to *S. mutans*/total whole salivary IgA concentrations and the total whole salivary IgA1 concentrations were found not to be significantly different between the caries-resistant individuals and the caries-susceptible individuals.

In dispute with previous studies, the levels of whole salivary IgA2 antibody to *S. mutans* levels were found to be relatively equal in the caries-resistant subjects as compared to the caries-susceptible subjects. Several explanations for this can be made. One explanation is that, as already reported, the actual proportions and concentrations of secretory IgA1 and IgA2 may shift in children from birth until adulthood is reached. Previous studies by Taubman and Smith[51] have shown that in children examined in the predentate

stage there were general population as well as individual fluctuations in IgA subclass levels.

Another explanation for this finding may be that antigenic cross-reactivity between IgA antibody to *S. mutans* and other bacterial species in the oral cavity exist and that the other bacteria are absorbing the IgA in whole saliva. A third explanation may be that since there is actually no reliable method to determine which subjects are currently exhibiting active or inactive caries progression in a cross-sectional study, then the actual clinical division of caries-resistant and caries-susceptible subjects cannot be determined. Attempts were made to divide the caries-susceptible group into active and inactive decay groups by determining the caries history based on clinical examination including oral exam and radiographs. The limited comparisons made were relatively equivalent but were not pursued based on the unreliable method of subject separation into caries-active and caries-inactive categories. The degree of caries activity could influence the type and number of bacteria present at the time of saliva collection, which would affect salivary IgA antibody levels. Another explanation for the findings is that the potential exists for

other microorganisms, which are cross-reactive with *S. mutans*, to be present in children in different numbers as compared to adult numbers.

More so with children than with other age groups, collection of saliva can sometimes be difficult due to variations in attention span, anxiety, and the ability to cooperate. Most children selected for this study were well behaved, willing patients but some lacked enough understanding and patience to remain comfortable for the period of time required for saliva collection. In some instances it was impossible to prevent external stimulation of saliva flow from the collection apparatus itself, but it was felt that every patient provided samples that would certainly maintain any guidelines put forward to this age range of subjects.

It is obvious in any clinical study that there is a wide fluctuation in environmental factors including such things as individual dietary habits and oral hygiene. These factors were controlled as well as possible by patient selection but may be better accounted for by using plaque to quantitate bacterial levels. Flow rates may be influenced by the time of day and anticipation of meals and activities which could decrease or increase the flow

rates. Flow rate is not considered constant but peaks with individual differences in relation to daily events. For this reason it was attempted to collect all samples in the morning hours but individual habits of meal times and activity levels may influence the outcome. Patient anxiety can also influence salivary flow rate. Some children are somewhat apprehensive about visiting the dentist no matter what the procedure. Approximately one half of the subjects for this study were placed in isolated, quiet rooms with little external distraction and the other half were placed in an open bay office setting with somewhat considerable external stimulation during the saliva collection. To what extent this may influence the saliva quality is unknown but it is suggested in the future to collect samples from all subjects under similar environmental standards. It was observed that patients left undisturbed in quiet room surroundings had an overall easier time with the collection apparatus and provided larger sample volumes although individual differences remained.

Two approaches were used to help correct any differences in flow rate between individuals. The first approach was the determination of the percentage *S. mutans*/total Streptococci for

each individual. The second approach was the determination of the total IgA/ml. This provided the equivalent information that would have been obtained from measuring the flow rate of each individual.

Two major influences on statistical significance are study design and sample size. Both of these factors were restricted due to time constraints. In the future a larger sample size would help establish a more reliable statistical evaluation. Cross sectional studies relate information in a more affordable and more timely manner, but it would be wise to conduct a combination cross sectional/longitudinal study in order to study individuals of varying ages with multiple sample analysis.

This investigation of the role of the immune response in natural protection against caries in children has clinical importance. This study indicates that the secretory immune response against *S. mutans* provides higher levels of resistance against dental caries in subjects with higher levels of sIgA. This study compared and documented the levels of IgA, IgA1 and IgA2 antibody to *S. mutans* for 7- to 11-year-old subjects so that a better understanding of fluctuation patterns in this age group could be obtained for future reference. This age range is important in that

it is the age of highest caries activity and these children would benefit the most from an enhanced immune response. As the data from this study indicate, the antibody levels to *S. mutans* can be used to identify those individuals who are at high risk for dental caries and they could be especially helpful in the 7- to 11-year age range. If those individuals at high caries risk could be identified then the possibility exists that their natural immune response could be enhanced (vaccination / immunization) resulting in a reduced caries rate. This could also provide information to clinicians that could initiate a more aggressive preventive program including the use of fluorides, pit and fissure sealants, and other preventive measures beyond the normal guidelines.

SUMMARY AND CONCLUSIONS

Developed countries have instituted programs to decrease dental caries such as water fluoridation, regular dental care, and increased awareness of contributing dietary factors. However, dental caries remains as the most common disease found in man. It is believed that if the body's immune system could be stimulated to produce more antibody to neutralize bacteria, then the caries rate could be reduced. It is thought that there is a relationship between the caries rate and the level of IgA2 antibodies to *S. mutans*. The purpose of this study was to determine the relationship between these two conditions and to make a comparison between caries-resistant and caries-susceptible children. This was performed by collecting parotid and whole saliva, quantitation of *S. mutans*, and ELISA analysis of total IgA concentrations and IgA antibody levels. It was found that the IgA antibody levels to *S. mutans* in whole saliva were significantly higher ($p < 0.05$) in caries-resistant subjects than the caries-susceptible subjects, but only slightly higher in parotid salivary IgA and whole salivary IgA1 antibody levels in comparison. It was also found that the IgA2 antibody

levels to *S. mutans* between the two groups were relatively equivalent. The conclusions from this study were that IgA antibody levels to *S. mutans* were higher in caries resistant subjects than in caries susceptible subjects but that a relationship could not be established between the IgA1 and IgA2 antibody levels to *S. mutans* and the degree of caries activity.

REFERENCES

1. Nowak A, Crall J. Prevention of dental disease. In: Pinkham, JR Pediatric dentistry: infancy through adolesence. Philadelphia: WB Saunders Co, 1988:37.

2. Smith GE. Tooth decay in the developing world: could a vaccine help prevent cavities. Perspect Biol Med 1988;31(3):440-53.

3. Diesendorf M. The mystery of declining tooth decay. Nature 1986;322:125-9.

4. Smith GE. Immunity and dental caries. Sci Prog 1987;69:429-38.

5. Ray A, Bertrand T, Chibber A, et al. World Development Report. Published for the World Bank. Oxford Univ Press, 1986.

6. Miller WD. The micoroorganisms of the human mouth. Phildelphia: S. S. White Manufacturing Co. 1890. Cited in: Loesche WJ. Role of *Streptococus mutans* in human dental decay. Microbial Rev 1986;50:353-80.

7. Clarke JK. On the bacterial factor in the etiology of dental caries. Br J Exp Pathol 1924;5:141. Cited in: McGhee JR, Michalek SM, Cassell GH, eds. Dental Microbiology. 1st ed. Philadelphia: Harper and Row, 1982:681-2.

8. Orland JF, Blayney JR, Harrison RW, et al. Experimental caries in germfree rats innoculated with enterococci. J Am Dent Assoc 1955;50:259-272.

9. Keyes PH. Research in dental caries. J Am Dent Assoc 1968; 76:1357-73.

10. Keyes PH. The infectious and transmissible nature of experimental dental caries: findings and implication. Arch Oral Biol 1960;1:304-20.

11. Fitzgerald RJ, Keyes PH. Demonstration of the etiologic role of Streptococci in experimental caries in hamsters. J Am Dent Assoc 1960;61:9-19.

12. Loesche WJ. Role of *Streptococcus mutans* in human dental decay. Microbial Rev 1986;50:353-80.

13. Loesche WJ, Rowan J, Straffon LH, Loos PJ. Association of *Streptococcus mutans* in human dental decay. Infect Immun 1975;11:1252-60.

14. Loesche WJ, Straffon LH. Longitudinal investigation of the role of *Streptococcus mutans* in human fissure decay. Infect Immun 1979;26:498-507.

15. Bratthall D. Demonstration of five serological groups of streptococcal strains resembling *Streptococcus mutans.* Odontol Revy 1970;21:143-52.

16. Perch B, Kjems E, Ravn T. Biochemical and serological properties of *Streptococcus mutans* from various human and animal resources. Acta Pathol Microbiol Scand 1974;82:357-70.

17. Coykendall AL. Base composition of deoxyribonucleic acid isolated from cariogenic streptococci. Arch Oral Biol 1970;15:365-8.

18. Michalek SM, Childers NK. Development and outlook for a caries vaccine. Crit Rev Oral Biol Med 1990;1(1):37-54.

19. Attstrom R, Egelberg J. Emigration of blood neutrophils and monocytes into the gingival crevices. J Periodont Res 1970;5:48-55.

20. Krasse B, Gahnberg L, Emilson CG. Effect of antibodies on colonization of gingival tooth surfaces by *Streptococcus mutans.* In: Lehner T, Cimasoni G, eds. The borderland between caries and periodontal disease. 3rd ed. Geneva: Editions Medecine et Hygiene, 1986.

21. Roitt IM, Lehner T, eds. Immunology of oral diseases, 2nd ed. Boston: Blackwell Scientific Publications, 1983:47-105.

22. Bienenstock J, McDermott MR, Befus AD, O'Neill M. A common mucosal immunologic system involving the branchus, breast, and bowel. Adv Exp Med biol 1978;107:53-9.

23. Mestecky J, McGhee JR, Russell MW, et al. Evidence for a common mucosal immune system in humans. Protides Biol Fluids Proc Colloq 1985;326:25-9.

24. Rudzik O, Perey DYE, Bienenstock J. Differential IgA repopulation after transfer of autologous and allogeneic rabbit Peyer's patch cells. J Immunol 1975;114:40-4.

25. Wagner M. Specific immunization against *Streptococcus faecalis*-induced caries in the gnotobiotic rat [Abstract]. Bacteriol Proc 1967;99.

26. McGhee JR, Michalek SM, Webb J, Navia JM, Rahman AFR, Legler DW. Effective immunity to dental caries: protection of gnotobiotic rats by local immunization with *Streptococcus mutans*. J Immunol 1975;114:300-5.

27. Taubman MA, Smith DJ. Effects of local immunizations with *Streptococcus mutans* on induction of salivary immunoglobulin A antibody and experimental dental caries in rats. Infect Immun 1974;9:1079-91.

28. Smith DJ, Taubman MA, Ebersole JL. Effect of local administration of glucosyltransferase antigens on experimental dental caries. Infect Immun 1979;26:83-9.

29. Taubman MA, Smith DJ. Effect of local immunization with glucosyltransferase fractions from *Streptococcus mutans* on dental caries in rats and hamsters. J Immunol 1977;118:710-20.

30. Smith DJ, Taubman MA, Ebersole JL. Preparation of glucosyltransferase from *Streptococcus mutans* by elution from water-insoluble polysaccharide with a dissociating solvent. Infect Immun 1979;23:446-52.

31. Taubman MA, Smith DJ, Ebersole JL. Antibody binding of glucosyltransferase enzyme preparations from homologous and heterologous serotypes of *Streptococcus mutans*. Adv Exp Med Biol 1978;107:317-25.

32. Taubman MA, Ebersole JL, Smith DJ, Reger RC. The immunochemical approach to dental caries prevention: secretory immune system adjuvant studies. J Dent Res 1980;59:2163-70.

33. Taubman MA, Smith DJ, Ebersole JL, Hillman JD. Immunological interference with accumulation of cariogenic microorganisms on tooth surfaces. In: Strober W, Hanson LA, Sell KW, eds. Recent advances in mucosal immunity. New York: Raven Press, 1982;371.

34. Van deRijn I, Bleiweis AS, Zabriskie JB. Antigens in *Streptococcus mutans* cross-reactive with human heart tissue. J Dent Res 1976;55:C59-C64.

35. Michalek SM, McGhee JR, Mestecky J, Arnold RR, Bozzo L. Ingestion of *Streptococcus mutans* induces secretory IgA and caries immunity. Science 1976;192:1238-40.

36. Michalek SM, McGhee JR, Babb JL. Effective immunity to dental caries: dose dependent studies of secretory immunity by oral administration of *Streptococcus mutans* of rats. Infect Immun 1978;9:217-28.

37. Michalek SM, Morisaki I, Harmon CC, Hamada S, McGhee JR. Effective immunity to dental caries: gastric intubation of *Streptococcus mutans* whole cells or cell walls induces protective immunity to gnotobiotic rats. Infect Immun 1983;39:645-54.

38. Cox DS, Taubman MA. Oral induction of the secretory antibody response by soluble and particulate antigens. Arch Allergy Appl Immunol 1984;57:75.

39. Kiyono H, Michalek SM, Mosteller LM, Torii M, Hamada S, McGhee JR. Enhancement of murine immune responses to orally administered haptenated *Streptococcus mutans*. Scand J Immunol 1982;16:455-63.

40. Ebersole JL, Taubman MA, Smith DJ. Adjuvants, glucosyltransferases and caries vaccine, In Glucosyltransferase, glucans, sucrose and dental caries. Washington, D.C.: IRL Press, 1983;241.

41. Morisaki I, Michalek SM, Harmon CC, Torii M, Hamada S, McGhee JR. Effective immunity to dental caries: enhancement of salivary anti-*Streptococcus mutans* antibody responses with oral adjuvants. Infect Immun 1983;40:577-92.

42. Loesche WJ. The bacterial etiology of dental decay and periodontal disease: the specific plaque hypothesis. In: Clark JW, ed. Clinical Dentistry. Philadelphia: Harper and Row, 1985:1-13.

43. Bratthall D. Selection for prevention of high caries risk groups. J Dent Res 1980;59:DII:2178-82.

44. Klock B, Krasse B. A comparison between different methods for prediction of caries activity. Scand J Dent Res 1979;87:129-39.

45. Delacroix DL, Dive C, Rambaud JC, Vaerman JP. IgA subclasses in various secretions and in serum. Immunology 1982;47:383-5.

46. Smith DJ, Taubman MA. Ontogeny of immunity to oral microbiota in humans. Crit Rev Oral Biol and Med 1992;3(1/2):109-33.

47. Smith DJ, King WF, Taubman MA. Isotype, subclass and molecular size of immunoglobulins in salivas from young infants. Clin Exp Immunol 1989;76:97-102.

48. Kohler B, Bratthall D. Intrafamilial levels of *Streptococcus mutans* and some aspects of the bacterial transmission. Scand J Dent Res 1978;86:35-42.

49. Carlsson J, Grahnen H, Jonsson G. Lactobacilli and Streptococci in the mouths of children. Caries Res 1975; 9:333-39.

50. Camling E, Kohler B. Infection with the bacterium *Streptococcus mutans* and salivary IgA antibodies in mothers and their children. Arch Oral Biol 1987;32:817-23.

51. Smith DJ, King WF, Taubman MA. Salivary IgA antibody to oral Streptococcal antigens in predentate infants. Oral Microbiol Immunol 1990;5:57-62.

52. Ganhnberg L, Smith DJ, Taubman MA, Ebersole JL. Salivary IgA antibody to glucosyltransferase of oral microbial origin in children. Arch Oral Biol 1985;30:551-6.

53. Ivanyi L, Lehner T. Interdependence of *in vitro* responsiveness of cord and maternal blood lymphocytes to antigens from oral bacteria. Clin Exp Immunol 1977;30:252-8.

54. Camling E, Gahnberg L, Krasse B. The relationship between IgA antibodies to *Streptococcus mutans* antigens in human saliva and breast milk and the numbers of indigenous oral *Streptococcus mutans*. Arch Oral Biol 1987;32(1):21-5.

55. Aaltonen AS, Tenovuo J, Lehtonen OP. Antibodies to the oral bacterium *Streptococcus mutans* and the development of caries in children in relation to maternal dental treatment during pregnancy. Arch Oral Biol 1988;33:33-9.

56. Mellander L, Carlsson B, Hansin LA. Secretory IgA and IgM antibodies to *E. coli* O and Poliovirus type I antigens occur in amnionic fluid, meconium, and saliva from newborns. A neonatal immune response without antigenic exposure: a result of anti-idiotypic induction? Clin Exp Immunol 1986;63:555-61.

57. Brandtzaeg P. The secretory immune system of lactating human mammary glands compared with other exocrine glands. Ann NY Acad Sci 1983;409:353-82.

58. Tank G, Storvick CA. Caries experience of children one to six years old in two Oregon communities (Corvallis and Albany). III. Relation of diet to variation of dental caries. J Am Dent Assoc 1965;70:393-403.

59. Camling E, Gahnberg L, Krasse B. The relationship between IgA antibodies to *Streptococcus mutans* antigens in human saliva and breast milk and the numbers of indigenous oral *Streptococcus mutans*. Arch Oral Biol. 1987;32:21-5.

60. Hughes M, Machardy S, Sheppard A, Langford D, Shepard W. Experiences in the development of a dental caries vaccine. Cited in: Hamada S, Michalek SM, Kiyono H, Menaker L, McGhee JR, eds. Molecular microbiology and immunobiology of *Streptococcus mutans*. Amsterdam, The Netherlands: Elsevier, 1986:349.

61. Gregory RL, Filler SJ. Protective secretory immunoglobulin A antibodies in humans following oral immunization with *Streptococcus mutans*. Infect Immun 1987;55:2409-15.

62. Gahnberg L, Krasse B. Salivary immunoglobulin A antibodies and recovery from challenge of *Streptococcus mutans* after oral administration of *Streptococcus mutans* vaccine in humans. Infect Immun 1983;39:514-9.

63. Cole MF, Emilson CG, Hsu SD, Li SH, Bowen WH. Effect of peroral immunization of humans with *Streptococcus mutans* on induction of salivary and serum antibodies and inhibition of experimental infection. Infect Immun 1984;46:703-6.

64. Mestecky J, Czerkinsky C, Brown TA, Prince SJ, Michalek SM, Russell MW, Jackson S, Scholler M, McGhee JR. Human immune responses to *Streptococcus mutans*. Cited in: Hamada S, Michalek SM, Kyiono H, Menaker L, McGhee JR, eds. Molecular microbiology and immunobiology of *Streptococcus mutans*. Amsterdam: Elsevier, 1986:297.

65. Taubman MA, Smith DJ. A mucosal approach to immunoprophylaxis of dental infections. Adv Exp Med Biol 1987;216B:1721-30.

66. Russell MW, Challachombe SJ, Lehner T. Specificity of antibodies induced by *Streptococcus mutans* during immunization against dental caries. Immunol 1980b;40:97-106.

67. Russell RRB, Abdulla E, Gilpin ML, Smith K. Characterization of *Streptococcus mutans* surface antigens. In: Hamada S, Michalek S, Kyiono H, Menaker L, McGhee JR, eds. Molecular microbiology and immunology of *Streptococcus mutans.* Amsterdam: Elsevier, 1986:61-70.

68. Russell RRB, Colman G. Immunization of monkeys (*Macaca fascicularis*) with purified *Streptococcus mutans* glucosyltransferase. Arch Oral Biol 1981;26:23-8.

69. Gregory RL, Michalek SM, Shechmeister IL, McGhee JR. Function of anti-*Streptococcus mutans* antibodies: anti-ribosomal antibodies inhibit acid production, growth, and glucose phosphotransferase activity. Infect Immun 1984;45:286-9.

70. Gregory RL, Kindle JC, Hobbs LC, Filler SJ, Malmstrom HS. Function of anti-*Streptococcus mutans* antibodies: inhibition of virulence factors and enzyme neutralization. Oral Microbiol Immunol 1990;5:181-8.

71. Curtiss R,III. Genetic analysis of Streptococcus mutans virulence and prospects for an anticaries vaccine. J Dent Res 1986;65:1034-45.

72. Lehner T, Russel MW, Caldwell J, Smith R. Immunization with purified protein antigens from Streptococcus mutans against dental caries in rhesus monkeys. Infect Immun 1981;34:407-15.

73. Lehner T, Challacombe S, Caldwell J. Immunological basis for vaccination against dental caries in monkeys. J Dent Res 1976;55:C166-C180.

74. Challacombe SJ, Lehner T. Salivary antibody responses in rhesus monkeys immunized with *Streptococcus mutans* by the oral, submucosal or subcutaneous routes. Arch Oral Biol 1980;24:917-25.

75. Riviere GR, Papagiannoulis L. Antibodies to indigenous and laboratory strains of *Streptococcus mutans* in saliva from children with dental caries and from caries-free children. Amer Aca Dent 1987;9(3):216-220.

76. U.S. Department of Health and Human Services; National Institute of Dental Research. Oral Health of United States children: national survey of dental caries in US school children., 1989. NIH Publication Number 89-2247.

77. Gold OG, Jordan HV, Van Houte J. A selective medium for *Streptococcus mutans*. Arch Oral Biol 1973;18:1357-64.

ABSTRACT

A COMPARISON OF IGA ANTIBODY LEVELS IN CARIES-RESISTANT AND CARIES-SUSCEPTIBLE CHILDREN

by

Paul Todd Rose

Indiana University School of Dentistry
Indianapolis, Indiana

Secretory immunity is believed to play a role in natural resistance to dental caries. Although dental caries has dramatically decreased in children in the United States, there remains a population of caries-susceptible children even in fluoridated communities. Previous studies have shown a positive correlation between salivary immunoglobulin A (sIgA) antibody levels to *Streptococcus mutans* and caries resistance in adults. In the present study, an enzyme-linked immunosorbent assay (ELISA) was

used to compare IgA antibody levels to *S. mutans* in saliva from 20 caries susceptible (DMFS greater than 5) and 20 caries-resistant (DMFS less than or equal to 1) children (ages 7-11). All subjects resided in fluoridated communities. Salivary *S. mutans* numbers were significantly higher ($p \leq 0.05$) in the caries susceptible (31.2 percent of total streptococci) group than in the caries resistant (1.6 percent of total streptococci) group. Whole saliva from caries-resistant children had significantly higher ($p = 0.05$) levels of IgA antibodies to *S. mutans* than saliva from caries-susceptible children. However, whole saliva from caries-resistant children had similar levels of IgA1 or IgA2 antibodies against *S. mutans* to saliva from caries-susceptible children. These results suggest that IgA antibody to *S. mutans* may play a role in natural protection from dental caries in children and confirm previous reports indicating a role for salivary IgA antibodies to *S. mutans* in mediation of caries.

CURRICULUM VITAE

Paul Todd Rose

June 21, 1964	Born in Charleston, West Virginia
May 1986	BS, West Virginia University Morgantown, West Virginia
May 1989	DDS, West Virginia University School of Dentistry Morgantown, West Virginia
July 1989 to July 1990	Dental General Practice Residency Charleston Area Medical Center Charleston, West Virginia
July 1990 to July 1991	Private Practice Beckley, West Virginia
July 1991 to July 1993	MSD Program, Pediatric Dentistry Indiana University School of Dentistry Indianapolis, Indiana
May 9, 1992	Married Rachel Joy Andrada

Professional Organizations

American Dental Association
American Academy of Pediatric Dentistry
International and American Associations for Dental Research